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Unraveling the Origins of Social Parasitism in *Megalomyrmex* Ants

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Unraveling the Origins of Social Parasitism in *Megalomyrmex* Ants

by

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Dedication

To Lubo Antonov,

for your love and unwavering support

To Milena Antonova,

for bringing perspective and balance during these final two years

To my Mom, Susan Adams, the toughest woman I know.

You have inspired me to take on life's challenges with fearlessness.

To Grandma Evelyn Cadle,

for providing a warm lap when I was young and encouraging creativity

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fascinating chemistry of ant venom. Jack instructed me in ant taxonomy in The Ant Course and invited me to join the ALAS team in Costa Rica where he introduced me to *M. mondabora*, *M. foreli*, and *M. modestus*. Without my undergraduate assistances I would not have accomplished as much work as I did. They helped me in all aspects of my work ranging from live ant care and collection to molecular, behavioral and experimental work. Many conducted independent projects and presented their work at UT and IUSSI conferences. I am not only thankful for their help but very proud of their accomplishments. Thanks to Nikhil Advani, Antony Archer, Sara Dewitz, Abbie Green, Lauren Lacey, Anita Manasiya, Jennifer Mir, Lauren Moore, Kaci Myrick, Diana Nguyen, Chris Rosel, Komal Shah, Lindsey Stewart, and Andrew Tseng. Finally, I want to mention the numerous specimen donors listed in the tables of each chapter, these donations were crucial to my work.

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Unraveling the Origins of Social Parasitism in *Megalomyrmex* Ants

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Rachelle Martha Marie Adams, Ph.D.

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Supervisor: Ulrich G. Mueller and Michael J. Ryan

Social parasitism, the exploitation of a society by other social organisms, has evolved independently numerous times within social animals. In this thesis, I integrate behavioral, evolutionary and chemical analyses to elucidate the evolution of social parasitism in *Megalomyrmex* ants. I examine host-parasite interactions in two *Megalomyrmex* species, identify venom alkaloids, and reconstruct the phylogenetic relationships between species. In Chapter 1, I analyze nest architecture and behavioral interactions between the ant host *Cyphomyrmex cornutus* and its parasite *Megalomyrmex mondabora.* This is the first detailed account of the natural history of this host and its social parasite. In Chapter 2, I report a one-year-long fitness experiment that tests whether *Trachymyrmex* cf. *zeteki* colonies suffer reduced fitness from an association with the social parasite *Megalomyrmex symmetochus*. I show that *M. symmetochus* parasites negatively impact host fitness though several mechanisms, including a) manipulation of the host worker grooming behavior; b) castration of host queens produced by the host colony, which then become workers; and c) reduction of garden size, host worker number, and host reproductive output. In Chapter 3, I determine that five venom alkaloids of *Megalomyrmex* are taxonomically informative to help differentiate cryptic species within the *M. mondabora* complex; new species in this complex need to be described in a future taxonomic revision. In Chapter 4, I reconstruct phylogenetic relationships of the genus

Megalomyrmex with DNA sequence information. I conclude that the genus is monophyletic and corroborate two of the four species groups proposed by Brandão (1990) in a previous morphological revision. I also find evidence in support of Darwin's Predation Hypothesis on the origin of social parasitism, which postulates that social-parasitic behaviors evolve from predatory behaviors. Lastly, I discuss promising future research directions on the evolution of social parasitism in the ant genus *Megalomyrmex*, which could serve as a model for the study of social parasitism in other lineages of social insects.

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Introduction

BACKGROUND

A eusocial insect society functions as a "superorganism" made of specialized individuals (i.e. nurse workers, guards, soldiers, reproductive queen) that are essential parts of the collective (Hölldobler & Wilson 1990). Large insect colonies and nests serve as a predictable source of diverse commensals, predators, and parasites. Some of the associates are other social insects and the consequence of combining two (or more) specialized societies is not always clear. There are few examples of two-species ant societies that share a single nest or a common set of trails in an apparent mutualism (*Crematogaster limata-Camponotus femoratus*; *Pachycondyla goeldii-Odontomachus mayi*) (Orivel *et al.* 1997; Lenoir *et al.* 2001). Most two-species societies have a parasitic relationship where the parasite 1) forages within the host colony and lives in it or nearby (i.e., xenobiosis / lestobiosis), 2) repeatedly raids host colonies to steal host brood (i.e., dulosis), 3) invades and produces predominantly or exclusively sexual offspring (i.e., inquilism), or 4) invades, produces workers, and eventually kills the host queen, resulting in colony takeover by the parasite (i.e. temporary social parasitism) (Lenoir *et al.* 2001). The common feature of all these diverse social-parasite types is that they boost their own fitness at the expense of host fitness.

Eusocial insect societies are remarkable model organisms in evolutionary biology due to their complex adaptations and associations (Beekman & Oldroyd 2008; Ratnieks & Wenseleers 2008). Each colony is characterized by a mixture of cuticular hydrocarbons that are shared between nestmates (Soroker *et al.* 1998). This mixture of hydrocarbons provides individuals with nestmate recognition cues that allow them to discriminate between nestmates and non-nestmates (Singer 1998; Lahav *et al.* 1999; Oppelt *et al.* 2008). Colony members guard the nest by using a "password system" made of these

nestmate recognition cues, just as immune systems protect the body in multicellular organisms against invasion by parasites and diseases. Commensals, predators, and parasites exploit the colony password system by mimicking host recognition cues (i.e., chemical mimicry) to gain access to abundant resources (Lorenzi *et al.* 2004).

Convergent infiltration strategies have evolved multiple times within the social parasites of ants, bees, and wasps (Fisher 1987; Sick *et al.* 1994; Lorenzi *et al.* 2004; Lambardi *et al.* 2007). In addition to "chemical mimicry", parasites can also use "chemical insignificance" (i.e., no odor at all) and/or "chemical weaponry" (Regnier & Wilson 1971; d'Ettorre *et al.* 2000; d'Ettorre & Heinze 2001; Lenoir *et al.* 2001; d'Ettorre *et al.* 2002). The parasite is considered chemically insignificant if it lacks a recognition profile or has significantly less hydrocarbons than its host (Lambardi *et al.* 2007). Chemical weaponry can be used as a behavioral disrupter (Regnier & Wilson 1971; Zimma *et al.* 2003) or a repellent (d'Ettorre *et al.* 2000) upon entering a host colony.

Social parasites in the ant tribe Solenopsidini are inquilines, xenobiotic, or lestobiotic. Most are workerless inquilines, dependent on the host to feed them and care for their brood (e.g., *Solenopsis daguerrei;* Calcaterra et al. 2000). Others are xenobiotic or lestobiotic, the former living with another ant society and the later living nearby the host (or in the nest walls). Both acquire food from the host colony but have a worker caste to care for their offspring (e.g., *Megalomyrmex symmetochus*, *Monomorium metoecus*, *Oxyepoecus bruchi*) (Wilson & Brown 1958; Hölldobler & Wilson 1990; de Albuquerque & Brandão 2004; Chapter 2). Also, social parasites can be either facultative or obligate associates (Hölldobler & Wilson 1990; Adams 2004) parasitizing species in the same genera as in *Solenopsis* and *Monomorium* parasites (Pitts et al. 2005) or in distantly related genera as in *Megalomyrmex* and *Oxyepoecus* (Adams 2004).

The ant genus *Megalomyrmex* (Formicidae, Solenopsidini) comprises 31 described species that are found from Southern Mexico to Argentina (Brandão 2003). Most species are free-living predators, whereas a small number form diverse associations with fungus-growing ant species. The fungus-growing or "attine" ants (Formicidae, Attini) are among the textbook examples of co-evolution and mutualism. The tribe is made up of over 230 described species, divided into the phylogenetically derived "higher attines" (which includes the leaf-cutting genera; and the phylogentically basal "lower attines" (Figure 1b,

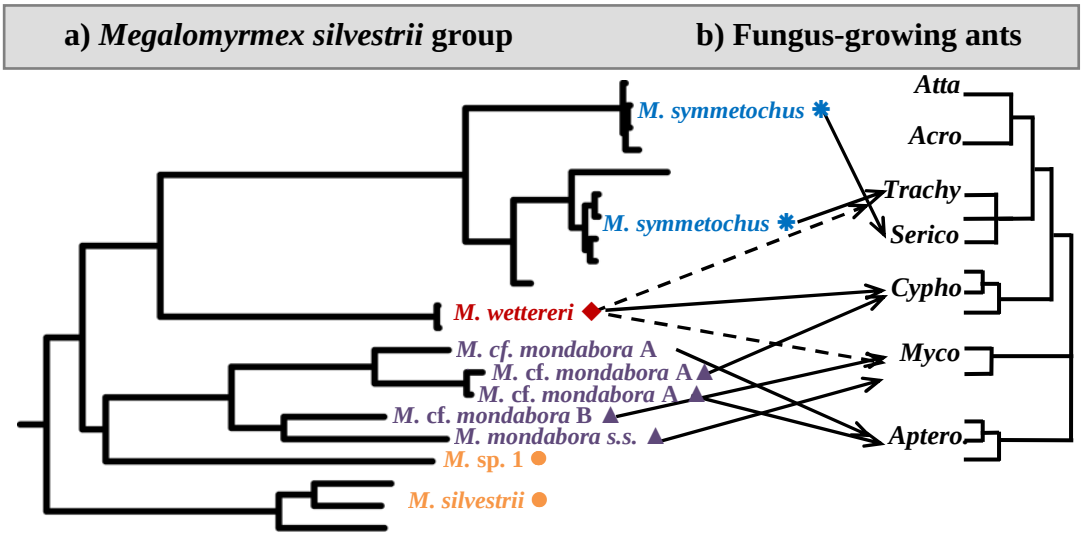

Figure 1:
a) Phylogenetic tree of *Megalomyrmex silvestrii* species group (adapted from Chapter 4). b) Host phylogeny of the "higher attines" (top two clades, of which the upper clade are the leaf-cutting ants) and "lower attines" (lower three clades) (adapted from Schultz & Meier 1995); arrows indicate to which genera the fungus-growing ant host species belong; Acro = *Acromyrmex*, Trachy = *Trachymyrmex*, Serico = *Sericomyrmex*, Cypho = *Cyphomyrmex*, Myco = *Mycocepurus*, Aptero = *Apterostigma*. c) Higher attine e.g. *Atta texana*. d) Lower Attine e.g. *Mycocepurus smithii*. Photos by Alex Wild.

c & d) (Mueller *et al.* 2005; Schultz & Brady 2008). The biology of the Attini involves not only their mutualistic association with a variety of fungal symbionts, but also specialized parasites and pathogens (Mueller 2002; Currie *et al.* 2003; Little & Currie 2008).

Megalomyrmex wettereri colonies are obligate agro-predators, aggressively attacking *Cyphomyrmex longicapus* host ants and usurping their nest, consuming fungus garden and brood (Adams et al. 2000). In contrast, *M. mondabora* and *M. symmetochus* are social parasites that live with their host, sharing the fungal resource and consuming host brood (see Chapter 1 & 2; Adams & Longino 2007). Each of these two *Megalomyrmex* species are currently thought to associate with several host species, some spanning both basal and derived attine genera (Brandão 2003; Adams & Dewitz 2006; Adams & Longino 2007; but see Chapter 3; Figure 1).

As in many predatory ant species (Hölldobler & Wilson 1990), chemical weaponry is likely used by free-living and parasitic *Megalomyrmex* species. Free-living *Megalomyrmex* are highly competitive at food sources just as their close relatives in the genera *Monomorium* and *Solenopsis* (Tribe: Solenopsidini) (Obin & Vander Meer 1985; Andersen *et al.* 1991; Adams personal observation). *Monomorium* "*rothsteini*" ants use venom alkaloids to repel competitors (Andersen *et al.* 1991) and it is possible that *Megalomyrmex* have similar potent chemical weapons. Free-living *Megalomyrmex wallacei* workers can overtake an artificial bait station occupied by thousands of *Pheidole* ants within a few minutes (Adams personal observation). The presence of a few *Megalomyrmex* workers at the bait caused the *Pheidole* ants to flee the food source, in most cases without direct contact between *Megalomyrmex* and *Pheidole* workers.

The *Megalomyrmex* associated with fungus-growing ants likely use a similar strategy of chemical deterrence. In the agro-predator *M. wettereri*, scouts enter a host cavity and quickly usurp the garden and nest with little resistance (Adams et al. 2000). *Megalomyrmex wallacei* and *M. wettereri* gaster flag during competition and usurpation emitting volatile chemicals from their sting (Figure 2). The parasite, *M. symmetochus* also uses gaster flagging during the initial interactions it has with its host queen but only when threatened (Chapter 2). Gaster flagging appears to be a "warning shot" for opponents that prompt a "flight" response. When used in combination with intense antennal tapping, as in *M. symmetochus*, the opponent's behavior becomes submissive, with aggression eventually subsiding. Venom alkaloids (Appendix A, Table A1) are likely involved in the dramatic behavioral effects seen in hosts and competitors of *Megalomyrmex*, giving *Megalomyrmex* the advantage.

Figure 2: *Megalomyrmex wettereri* gaster flagging

My dissertation work focuses on understanding social parasitism and its evolutionary origins. I examine the natural history of two *Megalomyrmex* species (i.e. *M. mondabora* and *M. symmetochus*) and their behavioral interactions with their host (Chapters 1 & 2). I also measure the fitness impact *M. symmetochus* has on its host (Chapter 2), analyze venom alkaloids from several *Megalomyrmex* species and utilize a subset as a chemotaxonomic tool (Chapter 3; Table A1), and with phylogenetic reconstruction, test species groups proposed by Brandão (1990) to elucidate the origins of social parasitism in the *Megalomyrmex* genus (Chapter 4).

SYNOPSIS

Using an integrative approach, I pioneered a model social parasitic ant system (Figure 3). By traveling to Peru, Ecuador, Brazil, Panama and Costa Rica, I observed 14 different *Megalomyrmex* species in the field and the laboratory. I inspected over a thousand host

colonies and determined that the likelihood of finding a host parasitized by *Megalomyrmex* range from 1% to 6% depending on the season and the species. I established reliable field sites in Costa Rica and Panama that allowed me to repeatedly collect three free-living species and three fungus-growing ant associates.

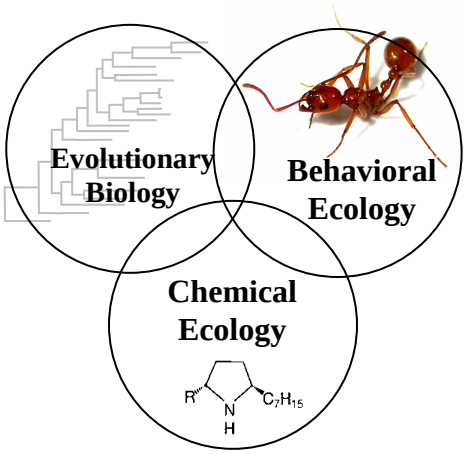

Figure 3: Three main areas of interest for my dissertation work

During multiple trips to Costa Rica, I investigated the extraordinary nesting habits of the fungus-growing ant *Cyphomyrmex cornutus* (Formicidae, Attini) and the natural history of *Megalomyrmex mondabora* (Formicidae, Solenopsidini) (Chapter 1). The *C. cornutus* nest is an oblong mass of accreted soil, attached to or suspended from low vegetation in wet forest understory. Less than a fourth of the nest volume has chambers and is inhabited by *C. cornutus*; the remainder is a semi-solid mass of soil often housing a variety of arthropods, including *M. mondabora* and unspecialized commensal ant species. *Megalomyrmex mondabora* appears to be a lestobiotic social parasite living peacefully near the host colony chamber, consuming host fungus garden and larvae.

In 2005, I collected parasitized and unparasitized colonies in Panama to set up a one-year experiment investigating the fitness impact *M. symmetochus* ants have on their host, *Trachymyrmex* cf. *zeteki*. *Megalomyrmex symmetochus* attacks the fungus-growing ant host by infiltrating host colonies, disrupting host hygienic behavior, and consuming colony resources (fungus garden, host brood). The presence of the social parasite reduced the garden mass, host worker-number, and host reproductive output compared to unparasitized control colonies, confirming that *M. symmetochus* is a parasite (i.e. xenobiont) rather than a mutualistic or commensal associate.

The venom alkaloids of most *Megalomyrmex* species that I collected were identified in collaboration with Dr. Tappey Jones from the Virginia Military Academy (Table A1). Some of these alkaloids are likely of great importance for host-parasite interactions and will stimulate future chemical ecology research determining venom alkaloid function (see Appendix B: Future Perspectives). Alkaloids are also useful in distinguishing cryptic species (Chapter 3). When integrating the alkaloid information with phylogenetic, morphological, and natural history information, it is apparent that the five alkaloids identified in *Megalomyrmex mondabora* samples are of chemo-taxonomic value. This work suggested that "*M. mondabora"* encompasses at least two cryptic species and is likely a species complex. Furthermore, as the phylogenetic analysis of the *Megalomyrmex* genus expands, it may be possible to determine the evolutionary pathways of all of alkaloids identified (Table A1).

I reconstructed the phylogenetic relationships of the genus *Megalomyrmex* by analyzing DNA sequence information of two genes with Maximum Likelihood and Bayesian methods. Although additional genes and species will be analyzed in the near future (see Appendix B), the current phylogeny prompts numerous exciting questions regarding cryptic species within the currently recognized M. *symmetochus*, *M. mondabora*, and *M. foreli* species (Brandão 1990, 2003). The fungus-growing ant associates were recovered as monophyletic, suggesting that social parasitism evolved once in the *Megalomyrmex* genus and is derived from the ancestral predatory species, supporting Darwin's Predation Hypothesis (Darwin 1859). The molecular data mostly agrees with Brandão's species groups based on morphology (Brandão 1990), although more samples are needed for two of the four species groups to fully address the monophyly of these groups.

The genus *Megalomyrmex* shows great promise to become a model system for the study of the evolution of behavior in eusocial insect societies. In this thesis, I integrate behavioral, evolutionary and chemical analyses to lay the groundwork for understanding

social parasitism (Figure 3). The emerging insights can now direct future work (e.g., chemotaxonomy, chemical ecology of infiltration strategies, evolution of alkaloids, and host choice driven sympatric speciation and reproductive isolating mechanisms) in the genus *Megalomyrmex,* which could serve as a model for the study of social parasitism in other lineages of social insects. The behavioral and/or physiological precursors needed for social parasitism to evolve are not fully understood, particularly in xenobiotic and lestobiotic solenopsidines. As additional social-parasitic systems are studied, a broad comparative perspective will eventually emerge.

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Chapter 1:

The nesting biology of the arboreal fungus-growing ant *Cyphomyrmex cornutus* and behavioral interactions with the social-parasitic ant *Megalomyrmex mondabora*

ABSTRACT

We describe the extraordinary nesting habits of the fungus-growing ant *Cyphomyrmex cornutus* (Formicidae, Myrmicinae, Attini) and the natural history of *Megalomyrmex mondabora* (Formicidae, Myrmicinae, Solenopsidini), a social parasite that inhabits nests of *C. cornutus* and other small attine ants. The study was carried out at two sites on the Atlantic slope of Costa Rica. The *C. cornutus* nest is an oblong mass of accreted soil, attached to or suspended from low vegetation in wet forest understory. Less than a fourth of the nest volume has chambers and is inhabited by *C. cornutus*; the remainder is a semi-solid mass of accreted soil often housing a variety of arthropods, including other unspecialized commensal ant species. Five *C. cornutus* colonies examined were parasitized by *M. mondabora.* Colonies of *M. mondabora* inhabited chambers very near those of the host. In laboratory observations, *M. mondabora* and *C. cornutus* workers interacted with little aggression despite the consumption of *C. cornutus* larvae and fungi by *M. mondabora*. During most interactions, *C. cornutus* workers responded submissively, whereas *M. mondabora* appeared indifferent or nonresponsive. *Megalomyrmex mondabora* parasitizes several other attine species (*Cyphomyrmex costatus, Cyphomyrmex salvini,* and *Apterostigma goniodes*), and it appears therefore a relatively unspecialized social parasite with broad attine host-association. The size of *M. mondabora* workers vary with host species, suggesting *M. mondabora sensu lato* comprises either cryptic species or the host environment affects worker size.

INTRODUCTION

The ant tribe Attini comprises the so-called fungus-growing ants, a group receiving increased attention as a model of coevolution and mutualism (Mueller, 2002; Currie et al., 2003; Mueller et al., 2005). The biology of the Attini involves not only their mutualistic association with a variety of fungal symbionts, but also a host of specialized predators and pathogens (Mueller, 2002; Currie et al., 2003). Among these are social parasites in the ant genus *Megalomyrmex* (Brandão, 1990; Adams et al., 2000a; Mueller et al., 2001). Heretofore the relationship between attine ants and *Megalomyrmex* has been difficult to study because the associations generally occur underground in small and cryptic attine nests. We describe here the unique arboreal nests of the attine ant *Cyphomyrmex cornutus* and the occurrence of the social parasite *Megalomyrmex mondabora* in those nests.

The genus *Cyphomyrmex* is one of the most abundant and species-rich genera in the tribe Attini (Bolton, 1995). Most *Cyphomyrmex* species are diminutive ants that nest in soil or leaf litter. Colonies are small, with tens to hundreds of workers. Workers in most species harvest insect feces (Figure 1.1e) and diverse vegetable debris (e.g., decaying flower parts) as substrate for their yeast garden. *Cyphomyrmex cornutus* is a highly distinctive species that occurs from Costa Rica to Ecuador (Kempf, 1968, Snelling and Longino, 1992). The only natural history information accompanying the species description is that the type specimens were found within rocky wet ravines (“quebrada”) and in lowland rainforest (Kempf, 1968). Snelling and Longino (1992) reported additional locality records but no new natural history information. Our field observations of *C. cornutus* reveal that it has a highly distinctive nesting biology that is unlike any other *Cyphomyrmex* species. The nests are large masses of accreted soil suspended in the low arboreal zone (Figure 1.1 a, b, & c). Colonies are large, with a thousand or more workers, and the nest structure houses not only the *Cyphomyrmex* colony but also many other

cohabiting ant species as well as other arthropods, including the social parasite *M. mondabora*.

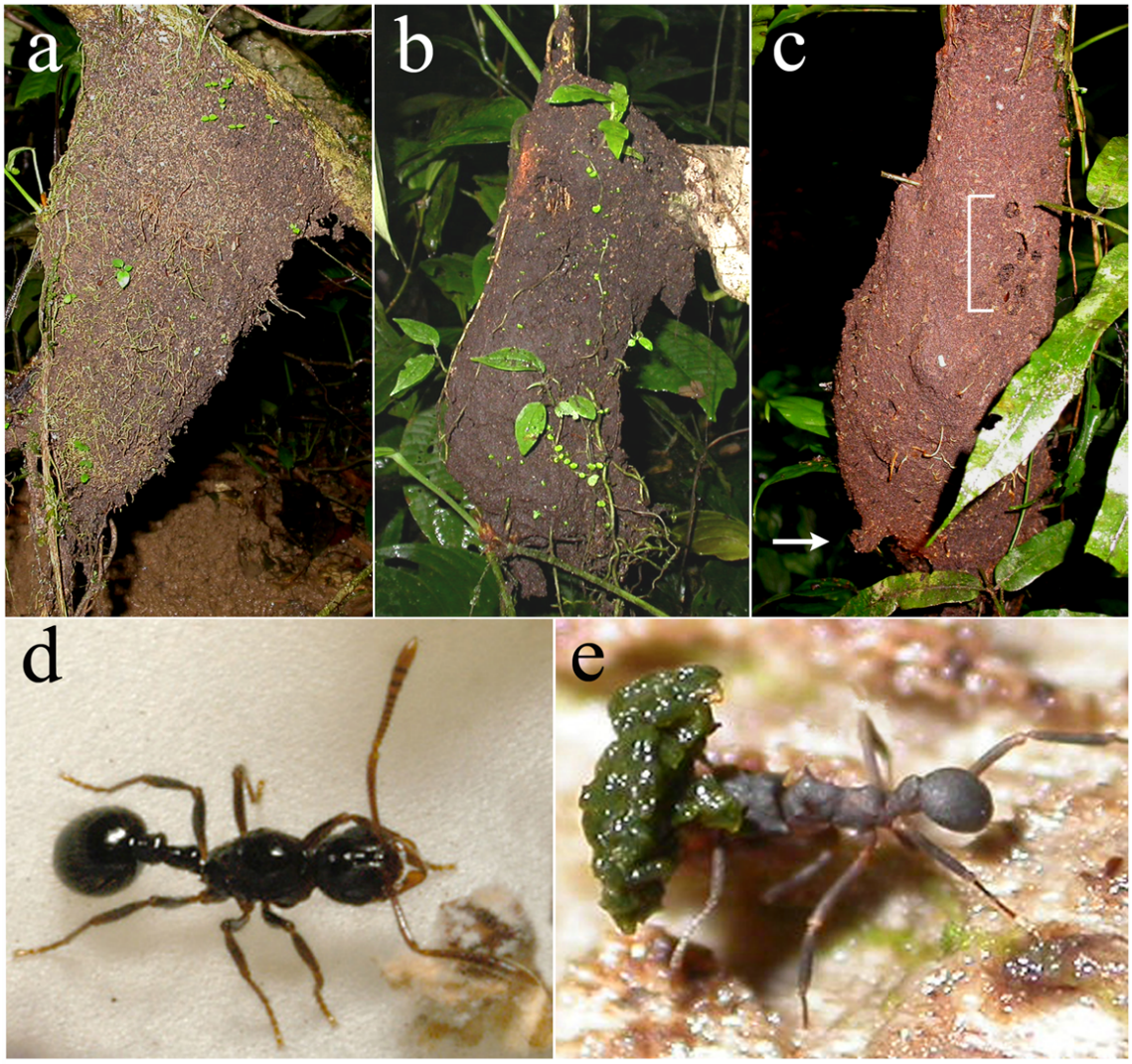

Figure 1.1: The three main nest types of *Cyphomyrmex cornutus* ants: (a) beard-shaped, (b) free-hanging, (c) attached. This nest was attached to the side of a small tree. Multiple entrances from the *C. cornutus* colony can be seen at the top (next to the bracket). The large turret at the bottom of the nest (see arrow) is the *M. mondabora* entrance. (d) *M. mondabora* queen (e) *C. cornutus* forager carrying insect frass.

Social parasitism has evolved multiple times in ants and other social hymenopterans (Buschinger, 1986; Hölldobler and Wilson, 1990; Carpenter et al., 1993; Cervo and Dani,

1996; Pedersen, 1996). Although in many cases they remain rare relative to the abundance of the host, social parasites can severely affect host colony fitness by exploiting key behaviors related to parental care or by consuming brood and/or food stores (Hölldobler and Wilson, 1990). The Attini suffer from social parasites and agro-predators (species that usurp the fungus garden from the attine ants) in the genera *Pseudoatta*, *Acromyrmex*, *Gnamptogenys*, and *Megalomyrmex* (Brandão, 1990; Adams et al., 2000a; Bekkevold and Boomsma, 2000; Brandão, 2003; Dijkstra and Boomsma, 2003; Boomsma, 2004). The first two are social parasites derived from ancestral fungus-growing ant lineages within the Attini, whereas both *Gnamptogenys* and *Megalomyrmex* originated independently in lineages distantly related to their attine hosts. The two *Pseudoatta* species are considered advanced social parasites in that they no longer have a worker cast and do not resemble their host (Hölldobler and Wilson, 1990; Delabie et al., 1993; Schultz et al., 1998). *Acromyrmex insinuator* is a less advanced social parasite that resembles its congeneric host and still retains a minimal worker cast. It is considered a transitional or incipient social parasite that is in the process of evolving more derived features (Hölldobler and Wilson, 1990; Schultz et al., 1998; Sumner et al., 2004). Unlike the *Gnamptogenys* and *Megalomyrmex* associates, the social parasites in *Acromyrmex* and *Pseudoatta* arose within the Attini.

Megalomyrmex is distantly related to Attini and belongs to the Solenopsidini, containing thief ants and fire ants (Bolton, 2003). The genus is made up of both free-living and social-parasitic species found in wet and subtropical forests from southern Mexico to Argentina (Brandão, 1990; 2003). The 31 known species have been divided taxonomically and behaviorally into the *leoninus*, *pusillus*, *modestus*, and *silvestrii* species groups (Brandão, 1990). Most species are unassociated with the fungus-growing ants and are free-living (non-parasitic). Eight species, classified in the *silvestrii* group, are assumed to be associated with fungus-growing ants (Brandão, 1990). These associations may be facultative or obligate and may involve brood predation and fungal (agro-)

predation (Adams 2000a; Brandão 1990; 2003). *Megalomyrmex wettereri,* an obligate agro-predator, aggressively attacks attine host ants and usurps their gardens and nest cavities (Adams et al., 2000a). *Megalomyrmex symmetochus* lives peacefully with its attine hosts and is an obligate social parasite (Wheeler, 1925). We report here that *M. mondabora* also appears to be an obligate social parasite living peacefully with its attine hosts, consuming host larvae and fungus-garden.

MATERIALS AND METHODS

John Longino (JTL) observed 11 separate nests of *C. cornutus* during multiple collecting trips to Costa Rica between 1985 and 2003. These nests were found on the Atlantic slopes of the Cordillera de Guanacaste, Cordillera de Tilarán, Cordillera Volcanica Central, and Cordillera de Talamanca. Two of these nests contained *M. mondabora*. Rachelle Adams (RMMA) carried out a survey of *C. cornutus* nests during February and August 2003 and June 2005 at two localities in Costa Rica. Both sites were on the Atlantic slope of Volcan Barva in the Cordillera Volcanica Central. One site was the Cascante Refuge (11km SE La Virgen, 450-550m, 10°20'N, 84°04'W), on the Barva transect in Braulio Carrillo National Park (Pringle et al., 1984; Hartshorn and Peralta, 1988). The other site was La Selva Biological Station (10°26'N, 84°00'W, 50m), 15km down slope from the Cascante Refuge (McDade et al., 1993). Both sites are tropical wet forest.

RMMA examined a total of 75 *C. cornutus* nests, 35 from Cascante (Feb. 2003) and 40 from La Selva (Aug. 2003). Nest location, shape, and physical dimensions were recorded. All nests at both sites were examined for the presence of *M. mondabora* and nests at La Selva were examined for other ant species on the surface. Nests were inspected in the field by first observing activity on the surface, searching entrances, and then carefully removing one side of the nest structure, which was removed by carefully scraping off layers with featherweight forceps. The nest material was sufficiently rigid to allow the

creation of a window into the nest chambers without nest collapse. Most of the solid soil mass at the bottom of the nest structure was left intact. In all but one case, the *C. cornutus* nests withstood the disturbance to their nest structure and repaired the damage within 24 hours. One colony moved their nest to a nearby site following examination.

The 40 *C. cornutus* colonies that were marked at La Selva in 2003 were reexamined in 2005 and classified as live, uninhabited, moved, or not found. Nest structures that were empty, dry, and crumbling were considered uninhabited. A few colonies were suspected of moving nearby because there was an active nest within 25cm of the old abandoned nest structure and containing a sizable colony. In a few cases flags for marking nests could not be relocated or were found with no remnants of the preexisting nest. These colonies either moved or were dead.

During the February 2003 survey, five nests were collected in their entirety. The five nests have collection reference numbers RMMA030213-09, AGH030212-13, RMMA030213-10, RMMA030212-07, and RMMA030213-07 but are referred to as nests one to five, respectively, in this paper. Nests #1, #2, and #5 were parasitized by *M. mondabora*. Nests #3 and #4 were unparasitized. Only Nests #1-3 were thoroughly dissected and all contents counted. All three nests contained additional ant species known to be arboreal ants with generalized nesting habits, facultatively present in or on the *C. cornutus* nests.

Five subcolonies were established in 100x15mm Petri dishes, using nesting material and 15-20 live workers per species from the collected nests. Four subcolonies contained yeast garden, *C. cornutus* workers and brood, and *M. mondabora* workers and brood. Subcolony A1 was from nest five, subcolonies B1, B2, and B3 from Nest #1. Subcolony C1 initially contained yeast garden, 15-20 *C. cornutus* workers and brood from Nest #4 (an unparasitized nest). The *C. cornutus* workers were allowed approximately four hours

to acclimate to the Petri dish, then four workers of *M. mondabora* from Nest #1 were introduced. Brief behavioral observations were made sporadically over four days.

In 2005, two additional colonies from the Cascante site (RMMA050627-01 and RMMA050625-01, hereafter referred to as Nests #6 and #7) were studied using video recordings one to four days after their collection. *Cyphomyrmex cornutus* nests were laid on their side and placed in clear nestboxes (10 x 10 x 20.5cm) with ½ cm of moistened plaster on the nestbox floor. The sides were painted with fluon to prevent the ants from escaping during observations. When ants were not observed, the nestbox was covered with a lid to retain moisture. Large roots, plants, and a portion of the soil were removed such that a flat layer of nesting substrate remained in the nest box. A large Petri dish (100x15mm) covered in red cellophane was provided as a refuge for either species. *Megalomyrmex* ants were observed by following them with the camera or keeping the camera stationary above the colony. Thirty-seven minutes and 24 seconds of Nest #6 and 33 minutes of Nest #7 were examined and 137 interactions were analyzed using slow and real time playback.

JTL, RMMA, and others made additional collections and qualitative observations on *M. mondabora* at multiple sites in Costa Rica and Panama. Worker size variation in *M. mondabora* was examined with respect to attine host.

RESULTS

Nest structure

Nests of *C. cornutus* are constructed of accreted soil. There are three main types of nest structures: beard-shaped, pendant, and attached (Figure 1.1a, b, & c). “Beard-shaped” nests hang vertically under a large branch, from near-horizontal surfaces (Figure 1.1a). “Pendant” nests hang by small vines (some less than a centimeter in diameter) (Figure

1.1b). “Attached” nests are broadly attached to a near-vertical surface, such as the base of an epiphytic plant or flat against a tree trunk or rock (Figure 1.1c). Nests are usually penetrated with plant roots from nearby vines and epiphytes. The ants sometimes incorporate live or dead leaves into the nest and place insect feces on the surface. The top portion of the nest (approximately the upper fourth) has multiple nest entrances (Figure 1.1c) and is filled with thin walled chambers built around roots, both speckled with the fungal garden. Large piles of beetle elytra, other insect fragments, and insect feces are incorporated into the fungus garden or are in close proximity. The fungus is the yeast form typical of *Cyphomyrmex* species in the *rimosus*-group, comprised of small polygonal masses that dot the surface of the substrate. The bottom portion of the nest is typically dense, with few passageways, and it is not inhabited by *C. cornutus*. There are often plant seedlings and/or mushrooms on the nest surface. These nests are not considered true “ant gardens” in the usual sense (Hölldobler and Wilson, 1990), because the plants never grow very large and they are not consistently present.

Cyphomyrmex cornutus colonies occur in both mature and secondary forest and are patchily distributed. They are often in very shaded and humid areas near ground level although the canopy was not surveyed in this study. At La Selva, *C. cornutus* is very abundant in second growth on the STR trail and rare or absent on most other trails. The average height of nests from the ground (measured from the base of the nest) was 120.2cm (n=37; range 71.1 - 205.7cm). The average length of the nest was 20.1cm (n=35; range 8.8 - 40.6cm), and the average width was 10.1cm (n=34; range 5.08 - 15.24cm). The average circumference, measured only for the pendant and beard-shaped nests, was 26.8cm (n=14; range 12.7 - 38.1cm) and the depth, only measured on a few “attached” nests, averaged 4.6cm (n=8; range 2.5 - 6.4cm). Eleven nests observed by JTL at various localities showed qualitatively similar patterns of nest size, location, and shape, although one nest was imbedded in a cavity in the trunk of a large *Xanthosoma* sp. (Araceae). In all cases the length of the nest exceeded the width, resulting in a vertical,

oblong shape. Whole colony counts of *C. cornutus* revealed that worker populations could be in the thousands and colonies are monogynous (Table 1.1).

Colony survival rate

In 2005, survival rates of the 40 colonies marked and measured in 2003 were determined. Of the 40 colonies observed in 2003 nine remained alive (22.5%), 22 were inactive (55%), seven could not be found (17.5%), and two appeared to have moved close by (5%).

Commensal associates and guest ant colonies

Eighteen out of 40 (45%) colonies examined at La Selva had additional ant species on the surface or subsurface of the nest (Table 1.2). A total of 10 species were collected, most of them single occurrences. However, *Pheidole flavens* was found on the surface or subsurface of 12 out of 40 colonies (30%) at La Selva. One unidentified *Pheidole* queen and hundreds of *Pheidole flavens* workers were found in one dissected colony from Cascante (Table 1.1). A small colony of *Pachycondyla bugabensis* and colonies of a small unidentified *Solenopsis* were found in each of two *Cyphomyrmex* nests dissected by JTL. The nest dissected by Ulrich Mueller also contained a *Brachymyrmex* nest (Table 1.1). Qualitative examination of various dissected or subsampled nests revealed an abundance of additional arthropod life in the peripheral and lower portions of the nest, including annelids, beetle larvae, nematodes, isopods, and silverfish. It is unclear how close or codependent the associations are between *C. cornutus* and these "guests." It is likely that many or all of these species will also be found unassociated with *C. cornutus*, and they are perhaps opportunistic inhabitants, able to nest in various microhabitats similar to *C. cornutus* nests.

Occurrence of *M. mondabora* in *C. cornutus* nests

Megalomyrmex mondabora occurred at low frequency in *C. cornutus* nests, and appeared to be more abundant at higher elevations. Two of the 11 (18.2%) *C. cornutus* nests examined by JTL contained *M. mondabora* colonies. RMMA's surveys revealed colonies of *M. mondabora* in three of 35 (8.6%) *C. cornutus* nests at Cascante in 2003 and two of five (40%) in 2005, and none in the 40 nests at La Selva. One of the three *M. mondabora* colonies collected at Cascante was first found by JTL in 2002, then was resurveyed by RMMA in 2003. It contained over 100 workers and many alate queens and males when discovered in June 2002, and in February 2003, the nest contained 249 workers and no alates (Table 1.1, nest 1). The two whole colony counts of *M. mondabora* revealed single queens and worker populations of about 250. Although the *M. mondabora* colonies were found in a chamber very close to *C. cornutus* nest chambers, they maintained a separate entrance and could be found on the nest surface or near the nest on the ground. The entrance varied in conspicuousness, from having a large turret shaped entrance (Figure 1.1c) to having no entrance superstructure.

Laboratory observations of host-parasite interactions

On establishment of the four subcolonies (A1, B1, B2, and B3), the *C. cornutus* and *M. mondabora* workers immediately segregated in the Petri dishes. The *M. mondabora* workers often stole and consumed host brood and yeast garden. There was one instance of aggression observed by a *M. mondabora* worker towards a *C. cornutus* worker. When the *C. cornutus* worker approached the *M. mondabora* group, a *M. mondabora* worker quickly bit the head of the host worker. Otherwise interactions between the social parasites and the hosts appeared nonaggressive.

To investigate the initial interactions between host workers and an unfamiliar parasitic worker, *M. mondabora* workers were introduced to naïve *C. cornutus* workers from an unparasitized colony (C1) and the subcolony was continuously observed for 46 minutes

following the introduction. Upon first contact, *C. cornutus* workers displayed aggression with open mandibles and a sudden jerking motion towards the *M. mondabora* workers. This aggressive display, described as "jumping" by Kweskin (2004), is also seen in related *Cyphomyrmex* species such as *C. costatus* (Kweskin, 2004), *C. muelleri* and *C. longiscapus* (RMMA personal observation). The *Megalomyrmex* workers responded to this aggression by touching the *C. cornutus* workers with their antennae. The *C. cornutus* workers then bowed their heads, tucked their antennae into their antennal scrobes, remained stationary for a few seconds, and then walked away. *Cyphomyrmex cornutus* workers reacted similarly following many interactions they had with the *M. mondabora* workers. Eventually the *M. mondabora* grouped separately from the host workers, as seen in the infected subcolonies described above.

In 2005, a more detailed analysis of host and parasite interactions was made for two additional parasitized *C. cornutus* colonies. As in 2003, both parasite and host colonies kept their brood, queen, and the majority of the workers isolated from one another. The *C. cornutus* workers maintained the separation by building a wall of soil and insect exoskeletons around the *M. mondabora* colony while *Megalomyrmex* workers helped shape it. Several *M. mondabora* ants wandered throughout the nestbox, often coming into contact with *C. cornutus* workers. One hundred thirty-seven interactions between *M. mondabora* and *C. cornutus* workers were analyzed. Contact was initiated by *M. mondabora* workers 47 times (34.3%), by *C. cornutus* workers 66 times (48.2%), and by both simultaneously 24 times (17.5%). The three responses of the host and/or parasite following an interaction were indifference (no response), submissive response, and "twitching." The submissive response, only seen in the host species, begins with a downward tilt of the head, antennal retraction into the scrobes, and a slight gaster tuck (in one case the observation was made from the side and the gaster was vibrating faintly).Twitching, also only seen in the host species, was a quick backward motion on the horizontal plane while the body curled further. This behavior was different than

jumping which is a forward motion only seen during parasite introduction to a naïve host. Twitching variably occurred following the submissive response. In all cases the *M. mondabora* reacted with indifference. The *C. cornutus* on the other hand were either indifferent (n=104), exhibited a submissive posture followed by motionlessness (n=7), or exhibited a submissive posture, became motionlessness and then twitched (n=26). The duration of the submissive posture (including twitching) ranged from 1.67s to 13.96s (mean 4.82) and the number of twitches they exhibited ranged from 2 to 17 (mean 5.19).

Host use and worker size variation in *M. mondabora*

Megalomyrmex mondabora occurs in Costa Rica, Panama, and Brazil (Brandão, 1990; RMMA personal observation). The type specimens of *M. mondabora* were collected by W. L. Brown near Turrialba, Costa Rica. They were collected from the fungus nest of a small unidentified *Apterostigma* species (Brandão, 1990). JTL collected *M. mondabora* three times: (1) nocturnal foragers on dead wood at Refugio Eladio, a site at 800m elevation on the Río Peñas Blancas on the Atlantic slope of the Cordillera de Tilarán (10°19'N 84°43'W); (2) a colony inside a nest of *C. cornutus* at Estación Pitilla, a site at 600m elevation on the Atlantic slope of the Cordillera de Guanacaste (10°59'N 85°26'W); and (3) a colony inside a nest of *C. cornutus* at Refugio Cascante, one of the sites of RMMA's surveys. RMMA found a total of six *M. mondabora* colonies at the Cascantae site. In 2003, a single *M. mondabora* queen was collected in a *Cyphomyrmex salvini* colony and three other colonies containing workers were collected in *C. cornutus* nests. In 2005, two additional colonies were collected with *C. cornutus* colonies. *Megalomyrmex mondabora* has also been found associated with *Apterostigma goniodes* and *Cyphomyrmex costatus* in Panama (RMMA personal observation).

The workers from these collections are all very similar in gross morphology and the diagnostic features of the species. Intracolonial size variation is small, but there is high inter-colony size variation, and this variation is related to host. Six records of *M.*

mondabora from *C. cornutus* have head length 0.87-0.96mm, three records from *Apterostigma goniodes* have head length 0.76-0.79mm, and one record from *C. costatus* has head length 0.67. The size of the *M. mondabora* workers parallels the amount of food available: *C. cornutus* workers are large and the fungus gardens are large, *A. goniodes* workers are about the same size as *C. cornutus* workers but the colony and fungus garden is much smaller, and both workers and fungus gardens of *C. costatus* are smaller than those of *A. goniodes*.

DISCUSSION

Our studies reveal a *Cyphomyrmex* species and a socially parasitic *Megalomyrmex* species with remarkable natural history and ideally suited for the study of social parasitism . Once a population is located, *Cyphomyrmex cornutus* foragers can be baited with ground corn or rice and nests can be easily found and monitored. Most nests are within reaching distance (120.2cm from the ground) and some can be collected by simply clipping the vines from which they are hung. Although fast moving for attine ants, the majority of the workers can be captured by this method. In addition, commensal and parasitic ant colonies can also be collected in their entirety, facilitating the study of the symbiotic community of these species.

Megalomyrmex mondabora colonies are specialist parasites of fungus-growing ants and they do not appear to nest independently, outside this association. Intensive collecting of the ant fauna at La Selva (Longino et al. 2002) and elsewhere on the Barva Transect has never yielded a *M. mondabora* colony unassociated with an attine host. Mike Kaspari's numerous samples of leaf litter ants from $1m^2$ plots in Panama has never yielded a *M. mondabora* nest (Kaspari, pers. comm.). *Megalomyrmex mondabora* workers can be found on the outside surface of the host's nest during the day and out scouting or foraging at night. It is possible that workers forage outside their host colony in order to meet nutritional requirements. Of the four *Megalomyrmex* species associated with the fungus-

growing ants (*M. silvestrii, M. symmetochus, M. mondabora,* and *M. wettereri*), *M. symmetochus* is the only other obligate social parasite (Wheeler, 1925; Kempf and Brown, 1968; Brandão, 1990, Adams et al., 2000a). *Megalomyrmex silvestrii* is a facultative parasite and *M. wettereri* is an agro-predator of one host and a social parasite of another (Brandão, 1990; Adams et al., 2000a; Brandão 2003). *Megalomyrmex mondabora* is the second species reported to eat host larvae, paralleling earlier reports on *M. wettereri* (Adams et al. 2000a; 2000b). Fungal and larval consumption suggests a parasitic relationship between *M. mondabora* and *C. cornutus*, although true fitness impact is still unknown.

The behavioral interactions reported here are not unique to *C. cornutus* but are found in other *Megalomyrmex* host species. When a *Cyphomyrmex longiscapus* colony is usurped by *M. wettereri*, the *C. longiscapus* will tuck their heads, just as described for *C. cornutus,* and will often curl up and play dead when being attacked (Adams et al. 2000a; 2000b). *Trachymyrmex* cf. *zeteki* also exhibits a submissive posture when *Megalomyrmex symmetochus* approaches (RMMA unpublished). The cause of this dramatic reaction seen across host genera is unknown. Like other Solenopsidini genera, *Megalomyrmex* produce venom alkaloids (Jones et al. 1982; Jones et al 1991). These and other behavioral cues might induce the submissive responses.

Host related worker size variation

Megalomyrmex mondabora populations, occupying different host species, do not vary greatly in morphology with the exception of size. This variation may be caused by environmental or genetic mechanisms, or both. The host nest environment may influence development of *Megalomyrmex* larvae, through quality or quantity of food. Alternatively, *M. mondabora sensu lato* may be comprised of cryptic species or races, such that genetically distinct populations or species are specialized on particular attine host species.

Multiple host species leading to isolation and eventually speciation may be tested in several *Megalomyrmex* species (*M. mondabora*, *M. symmetochus*, *M. silvestrii*, and *M. wettereri*). *M. mondabora* parasitizes two yeast and two mycelium growing attine species in two genera (*C. cornutus*, *C. salvini*, *C. costatus*, and *Apterostigma goniodes*). *Megalomyrmex symmetochus* parasitize at least three species (*Trachymyrmex* cf. *zeteki*, *Sericomyrmex amabilis*, and a smaller *Sericomyrmex* species) (Wheeler 1925; Brandão 2003) and *M. silvestrii* parasitizes four species from four genera (*Apterostigma* sp. 1, *C. costatus*, *Sericomyrmex* sp. 1 and *Trachymyrmex* sp. 1) (Weber 1941; Kempf and Brown 1968; Brandão 2000; RMMA personal observation). Finally, *M. wettereri* parasitize *Trachymyrmex bugnioni* and are agro-predators of *Cyphomyrmex longiscapus*, usurping the garden rather than cohabiting with the host (Adams et al. 2000a; Brandão 2003). It would be interesting to know if all *Megalomyrmex* species that associate with multiple host species also show size variation and are genetically isolated. DNA sequence data and other biochemical characteristics (e.g., cuticular hydrocarbons and venom gland alkaloids) may also help differentiate populations or closely related species (Vander Meer et al., 1985).

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Nest ID and association	Ant species	Workers	Dealate queens	Alate queens	Alate Males
Nest #1					
Host	*Cyphomyrmex cornutus*	2657	1	0	0
Social parasite	*Megalomyrmex mondabora*	249	1	0	0
Commensal	*Solenopsis* JTL-005	4	3	0	0
Commensal	*Strumigenys biolleyi*	>20	0	0	0
Commensal	*Pachycondyla* cf. *villosa*	5	0	0	0
Nest #2					
Host	*Cyphomyrmex cornutus*	208	1	0	9
Social parasite	*Megalomyrmex mondabora*	260	1	0	12
Commensal	*Odontomachus hastatus*	0	1	0	0
Commensal	*Pheidole sp.*	0	1	0	0
Commensal	*Pheidole flavens*	>100	0	0	0
Nest #3					
Host	*Cyphomyrmex cornutus*	1105	1	10	0
Commensal	*Crematogaster longispina*	>20	0	0	0
Commensal	*Hypoponera JTL-007*	1	1	0	0
Commensal	*Pachycondyla lineaticeps*	9	2	0	0
Commensal	*Paratrechina steinheili*	18	0	0	0
Commensal	*Pheidole JTL-052*	8	0	0	0
Commensal	*Solenopsis picea*	1	0	0	0
Commensal	*Strumigenys biolleyi*	1	0	0	0
UGM020604-07					
Host	*Cyphomyrmex cornutus*	4117	0	190	138
Commensal	*Brachymyrmex sp.*	?	?	?	?

Table 1.1: Colony counts, including commensals and social parasites of four *C. cornutus* colonies, two of which are parasitized by *M. mondabora*. Nests one to three were collected in February 2003 at the Cascante Refuge on the Barva Transect, Braulio Carrillo National Park, Costa Rica. Nest UGM020604-07 was collected by U. Mueller in 2002, on Punta Pena Road, Panama (commensal ant workers were not counted).

Commensal ant species on nest surface	Number of *C. cornutus* nests
Apterostigma collare	1
Brachymyrmex JTL-007	2
Pheidole anastasii	1
Pheidole flavens	12
Pheidole perpusilla	1
Pyramica alberti	1
Solenopsis geminata	1
Solenopsis picea	1
Strumigenys micretes	1
Wasmannia auropunctata	2

Table 1.2: Commensal ant species found associated with 18 out of 40 *C. cornutus* nests at La Selva Biological Station, Costa Rica. Commensal associates were collected from the surface and subsurface of *C. cornutus* nest structures. Numbers indicate the number of nests on which each species was found.

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Chapter 2:

Fungus-growing ant host fitness is reduced by the presence of a social parasite

ABSTRACT

The social parasite *Megalomyrmex symmetochus* attacks the fungus-growing ant *Trachymyrmex* cf. *zeteki* by infiltrating host colonies, disrupting host hygienic behavior, and consuming colony resources (fungus garden, host brood). In mature host colonies, female host reproductives are mutilated (wing-clipped) by *M. symmetochus* workers, whereas host males are not attacked. Wing-clipped females become workers, and the mutilation of host females by the parasites therefore converts host reproductive effort to colony maintenance and growth. In a one-year experiment, the presence of the social parasite reduced the garden mass, host worker-number, and host reproductive output compared to unparasitized control colonies, confirming that *M. symmetochus* is a parasite rather than a mutualistic or commensal associate. This is the first study to successfully infect host colonies with social parasites and measure the fitness cost of social parasitism.

INTRODUCTION

In the entangled web of species interactions, the effects that species have on each other can range from parasitic to commensal to mutualistic. A parasite, by definition, increases its own fitness while decreasing the fitness of its host (Price 1980). Hosts can suffer directly through decreased survival (Schwanz 2008). However, some parasites have sublethal effects on their hosts by compromising physiological efficiency (e.g., digestion in mice) (Munger & Karasov 1989), reducing body size (e.g., wasp offspring) (O'Neill *et al.* 2007), altering offspring sex ratio (Kankova *et al.* 2007), or depressing reproductive output (Deter *et al.* 2007).

Eusocial insect societies face parasitic infection collectively, protecting their nestmates and ultimately their queen, the key individual responsible for colony reproduction (Schmid-Hempel 1989; Boomsma *et al.* 2005; Cremer *et al.* 2007). Parasites can influence the host colony by altering host worker behavior, causing a reduction in colony resources (i.e., food and workers), or killing current or future queens (Hölldobler & Wilson 1990). One of the most remarkable yet least understood symbioses is social parasitism, in which one social species serves as host of a second social species (Hölldobler & Wilson 1990). The consequence of combining two (or more) specialized societies is not always clear. Some of these two-species societies share a single nest and a common set of trails and appear mutualistic (*Crematogaster limata-Camponotus femoratus*; *Pachycondyla goeldii-Odontomachus mayi*; (Orivel *et al.* 1997; Lenoir *et al.* 2001). Most other two-species societies have a parasitic relationship where the parasite 1) forages within the host colony and lives in it or nearby (i.e., xenobiosis / lestobiosis), 2) repeatedly raids host colonies to steal host brood (i.e., dulosis), 3) invades and produces predominantly or exclusively sexual offspring (i.e., inquilism), or 4) invades, produces workers, and eventually kills the host queen, resulting in colony takeover by the parasite (i.e. temporary social parasitism) (Lenoir *et al.* 2001).

Based on our current knowledge of the biology of *M. symmetochus*, we hypothesize hypothesize that *M. symmetochus* is a social parasite. We report the results of a one-year study testing this hypothesis, examining the relationship between two cohabiting ant species, *M. symmetochus* a putative social parasite, and their host *Trachymyrmex* cf. *zeteki*. We specifically study infiltration and cohabitation behavior and the cost of the presence of the putative social parasite on host colony resources (i.e. workers and food stores) and reproductive output.

MATERIALS AND METHODS

Study System

The genus *Megalomyrmex* (Formicidae, Solenopsidini) comprises 31 described species (Brandão 2003 and references therein). Most are free-living predators, whereas a small number are assumed to be social parasites of fungus-growing ants (Brandão 1990). As far as is known, host and social parasites care for their own, separate broods. In addition, the parasitic *Megalomyrmex* produce a worker caste, suggesting a less derived relationship between the host and parasite species, compared to parasites that have lost the worker caste (Hölldobler & Wilson 1990). *Megalomyrmex symmetochus* appear to be obligately associated with fungus-growing ants and can be found in young and mature *T.* cf. *zeteki* colonies (RMM Adams pers. obs.). Mature host-parasite colonies can have nearly 100 parasite workers and hundreds of host workers. Colonies can live for at least two years in the laboratory and likely many more in the field (RMM Adams pers. obs.; Wheeler 1925).

The fungus-growing or ‘attine’ ants (Formicidae, Attini) are among the textbook examples of co-evolution and mutualism. Made up of over 230 described species, the attines maintain a mutualistic association with a variety of fungal symbionts, along with a number of specialized parasites and pathogens (Schultz & Brady 2008). They are faced

with unique challenges subject to all social insects and partners in close mutualistic relationships (Yu 2001; Poulsen *et al.* 2006; Cremer *et al.* 2007). They need to protect themselves from predators and parasites, and they must also bear the burden of protecting a fungal symbiont (Currie & Stuart 2001) with which they have coevolved (Chapela *et al.* 1994; Mueller 2002). Garden cultivars have become dependent on the ants, who supply substrate for consumption and who weed fungal contaminants that curb fungal growth (Weber 1972; Currie & Stuart 2001; Little *et al.* 2003). In turn, the ants rely on the fungal garden as a food source (Weber 1972; Quinlan & Cherrett 1979; Silva *et al.* 2003). *Megalomyrmex* ants attack both partners in this mutualism, consuming not only the fungus garden but also the ant larvae (Adams *et al.* 2000). These larvae are young workers or reproductives, and their elimination indirectly or directly impacts the fitness of the colony, respectively.

Colony collection

Colonies used in the main study were collected between July 21st to August 18th 2005 (two other colonies were collected in 1999, see observational results) in the forests off Pipeline Road, Soberanía National Park, Republic of Panamá. They were found in primary and older secondary rainforest along creek embankments and on steep slopes nearby. *Trachymyrmex* cf. *zeteki* colonies are easily located by their characteristic auricle-shaped nest entrance (Fernandez-Marin *et al.* 2004), although parasitized and unparasitized colonies are indistinguishable before excavation. First-year colonies established after mating flights during the early rainy season in 2005 (approx. May or June) were collected in small five dram vials, then transferred to small 60x15mm diameter Petri dishes lined with a ring of moistened cotton to increase nest humidity, and later transferred to permanent nest-boxes (see below).

Of the 250 queen-right first-year *Trachymyrmex* cf. *zeteki* colonies inspected in the field, fifteen (6%) contained *Megalomyrmex symmetochus* queens. Of the 15, four (26.7%) did

not contain host queens (possibly because the *Trachymyrmex* queen was foraging or died), and two (13.3%) had two parasite queens per colony. Sixty-five colonies of the 250 inspected were collected live (15 parasitized and 50 unparasitized).

At the start of the experiment, colonies were randomly chosen from the unparasitized colonies collected, and 44 colonies with 0-10 host workers (at the time of collection), were used. Experimental colonies were arranged according to the following three treatments. In a '*Megalomyrmex* Minus' colony (M-), the social parasite queen(s) was removed from a colony that was naturally parasitized before collection, leaving the host colony unparasitized (n = 11). In a parasitized or 'New Host' colony (NH), a social-parasite queen was removed from her original host colony (M-) and introduced into a naïve colony that had been collected parasite-free and presumably never had contact with a *Megalomyrmex* queen until experimental introduction (n = 17). Finally, 'Complement Control' colonies (CC) were unmanipulated, parasite-free colonies and were assumed to never have had contact with a *Megalomyrmex* queen (n = 17). The original gardens were removed and replaced with the same fungal strain from colony RMMA050105-29 to control for fungal strain performance. When M- colonies were queen-right (contained a queen), replicates consisted of all three nest types (NH, M-, CC) and were standardized for worker number and garden mass. Due to mortality, replicates broke down and CC and M- were combined into a control category (for analysis results see Appendix Table S2.1 in Supplementary Material). Treatments will be referred to as "Control" and "Parasitized" hereafter.

Colony maintenance

Colonies were maintained in square plastic (7.5 x 2 cm) nest-boxes with moist plaster bottoms connected to a dry foraging and refuse chamber of the same size. The ants were given a mix of UV-sterilized pecan catkins, and sterilized organic oats and polenta, to feed their fungus garden *ad libitum*. Unused substrate was removed from the foraging

chamber every two weeks. Within the nest chamber, garden was grown in a 60x15mm Petri dish, which could be removed with the garden for biweekly weighing. When the garden filled the dish, a new nest-box with Petri dish was attached and a small amount of garden was moved to the dish, stimulating use of this second nest chamber by the ants. Discarded fungus-garden was removed immediately to discourage fungal contaminate growth in the nest chamber. Pellet piles were allowed to accumulate in the nest or refuse chamber as they do in nature (see Little *et al.* 2003), then removed every two weeks. Occasionally, gardens would die or become depleted during the data collection period; rather than eliminating the colony from the experiment, a new garden was substituted at the original weight given at the start of the experiment (see Appendix S2.1). Colonies remained in dark conditions except during data collection and general maintenance. They were kept at room temperature approximately 20-23° Celsius and systematically rotated on the lab bench every two weeks to control for laboratory artefacts.

Data collection

Data were collected every two weeks beginning on November 7th 2005, when colonies stabilized and gardens were routinely fed and successfully cared for by the ants (41 days after colonies were moved to their permanent nest-boxes). Data were analyzed using JMP, version 4, SAS Institute Inc. (Sall 1989-2000) and parametric (one-tailed Student's *t*-test) and non-parametric (Wilcoxon rank-sum test) analyses (see Table S2.2) between independent groups were used where appropriate. A one-tailed Student *t*-test was used because we made an *a priori* decision to test for the cost of parasitism (i.e., we expected that the parasite negatively affected host fitness). All normal data were tested for equality of variances (Levene's Test) (see S2.2).

Garden weight

Garden and ant (brood and adult) mass (mg) was weighed together on a Petri dish of known weight in which the garden grew. Because of garden replacement events (gardens

were replaced back to their original weight if they died), the garden 'growth ratio' was analyzed. The growth ratio was calculated by subtracting the current garden weight from the previous garden weight (i.e., change in garden weight between two weeks), divided by 'previous garden weight'. This ratio (referred to as garden growth or garden weight change ratio, hereafter) was then averaged for each treatment in two week intervals and for the entire year. Treatment differences were assessed with a one-tail *t*-test and Wilcoxon rank-sum test and tested for unequal variances.

Worker and reproductive number

Live ants were quantified by counting the number of workers in a colony three times in close succession, then averaging the three counts. These counts became less accurate as the garden grew larger because ants working inside the garden become less visible. The total live and dead workers and reproductives were counted every two weeks for one entire year, except in four cases in which a two-week count had to be skipped because of logistical reasons (Figure 2.2b). Observations were averaged for each treatment per observation period, then for the entire year of data collection. Treatment differences were assessed with a one-tail *t*-test and Wilcoxon rank-sum test and tested for unequal variances. Total live workers at the end of the experiment were also averaged and analysed in the same way. Too few colonies produced reproductives at one year, therefore data were collected again seven months later and a Wilcoxon rank-sum test was performed.

Behavioral Observations

This experimental setup offered unique opportunities to observe behavioral interactions between the host and parasite ants beginning with parasite introductions. The parasite was introduced by first mixing the original and new host fungus garden in a new Petri dish to help blend garden related nest odors. Then the parasite queen was introduced to host workers (1-3 at a time). Finally, the host queen was placed in the Petri dish. Parasite

introductions were video-recorded for 10 colonies until aggression subsided or the introduction was reported a failure. In addition, general observations were made during the experiment and five Parasitized colonies were video-recorded for 30 minutes (February 16th to March 17th 2007).

RESULTS

Behavioral Interactions and Natural History

To ensure the highest rate of successful introductions, parasite queens were introduced to their new host colonies gradually, starting with the host workers. Surprisingly, the parasite queens were not attacked by the host workers. However, there was obvious aggression between the host queen and the parasite queen. The host queen attacked the parasite 1-8 times during the introduction process (observations lasting up to 160 minutes over several days). The parasite queen responded to aggression with intense antennal tapping that often led to host queen submission indicated by the tucking of her head and cessation of aggression. This submissive host behavior is similarly found in the fungus-growing ant workers *Cyphomyrmex cornutus*, when parasitized by *Megalomyrmex mondabora* (Adams & Longino 2007). In cases in which the host queen escalated her aggression, the parasite would wave her abdomen up and down (i.e., gaster flag) (in 62.5% of the 10 colonies observed), likely emitting volatile chemical compounds from her venom gland (Obin & Vander Meer 1985). *Megalomyrmex* have a nonpenetrating flat brush-like sting (i.e., spatulate) that allows them to hold a droplet of venom at the tip during gaster flagging and apply venom to the integument of aggressors (Jones *et al.* 1991). If the host queen continued to attack, the parasite queen retaliated by brushing venom on the host queen's body, which initiated a distressed response (quick abrupt running and floor rubbing). This only occurred in two unsuccessful introductions where the host queen died. Successful introductions were those in which aggressive behavior

eventually ceased (between 40 to 160 minutes after the first contact with the host queen) and the two queens coexisted peacefully.

Once colonies reached a moderate size (5-38 workers), interactions between the host workers, parasite workers, and the parasite queen were observed in a single 30 minute period in five colonies. Host workers were observed grooming the parasite workers and queen with a tendency to groom the abdominal area of the parasite more than the head and thorax. During this time, the parasite held her antennae in a backward submissive position. During additional sporadic observations, host workers were also seen on two occasions to place small fragments of fungus garden on the parasite queen while she remained still.

The parasites rarely initiated interaction with the host; however, in two colonies collected in 1999 (RMMA990929-06 and RMMA990928-04), parasite workers attacked their host two years after collection. In two parasitized *T.* cf. *zeteki* colonies with approximately 100-200 workers, *M. symmetochus* workers were observed chewing the wings off host female reproductives (Figure 2.1). A few workers would begin the attack but then one would slowly chew the wings either at the base or in the middle of the wing until they were fully or partially removed. All females present were attacked (approx. 4-10) and males were left alone (approx. 4-10).

Figure 2.1: Parasite workers wing-clipping and thus castrating a reproductive female in a mature host colony. A parasite worker mutilates the wings of a host female, resulting in stubby, non-functional wings (insert). Wing-clipping prevents reproductive females from dispersing and they become workers in their native colony.

The progression of parasite colony growth was also monitored. Like *Megalomyrmex wettereri* ants (Adams *et al.* 2000), the *M. symmetochus* queens build a fungal cavity in the host garden, where she remains most of the time with her brood. Four months after the start of the experiment, two parasite colonies produced their first workers, at six months three colonies had 1-2 workers, at nine months 2 colonies started to produce males, and at eleven months four colonies produced males. By the end of the experiment (12 months), all *Megalomyrmex* colonies produced males and four of the five produced 3-16 workers. The colony with the highest number of workers (16) was in a host nest (17NH) with the largest garden mass (2326mg) compared to the colony that did not produce workers (10NH; 67.3mg).

Colony growth: Garden and Workers

Colony growth was measured by the number of workers produced and by garden growth. Because of colony mortality during the experiment, sample sizes were reduced to 11 Control and five Parasitized colonies. Records for garden weight and total workers produced were averaged every two weeks to illustrate the difference between Parasitized and Control colonies (Fig. 2). After one year, the garden growth (*t*-test: DF 14 = 2.452, *P* = 0.014) and total workers produced (*t*-test: DF 14 = 1.712, *P* = 0.05445) were analyzed (Figure 2.3a). The number of workers alive at the end of the experiment (*t*-test: DF 14 = 1.709, *P* = 0.05475) was also marginally significant between treatments (see Table S2.2 for results of equal variance tests and Wilcoxon rank-sum test).

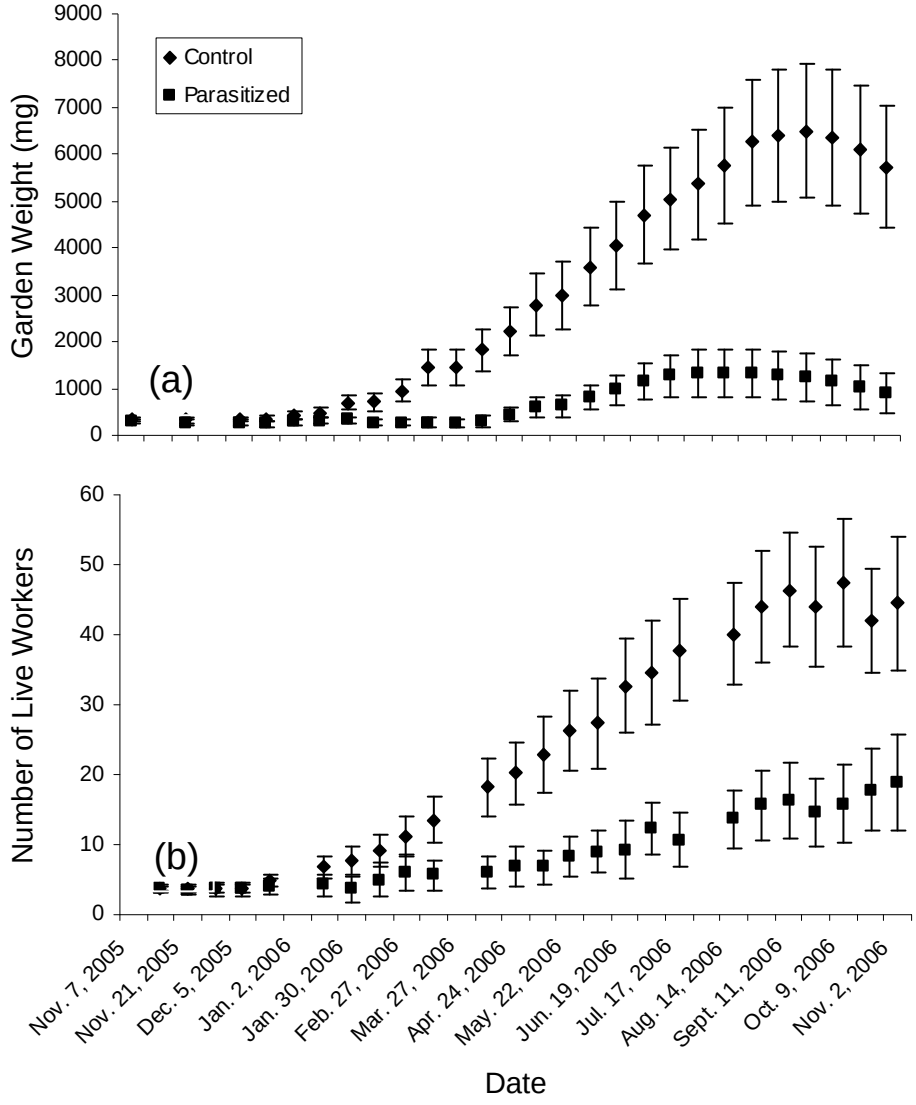

Figure 2.2: a) Average garden weight (mg) and b) live workers censuses every two weeks. Diamond markers represent Control colonies (n = 11) and square markers Parasitized colonies (n = 5). Error bars indicate standard errors. These graphs illustrate the depression of host colony growth caused by parasitism.

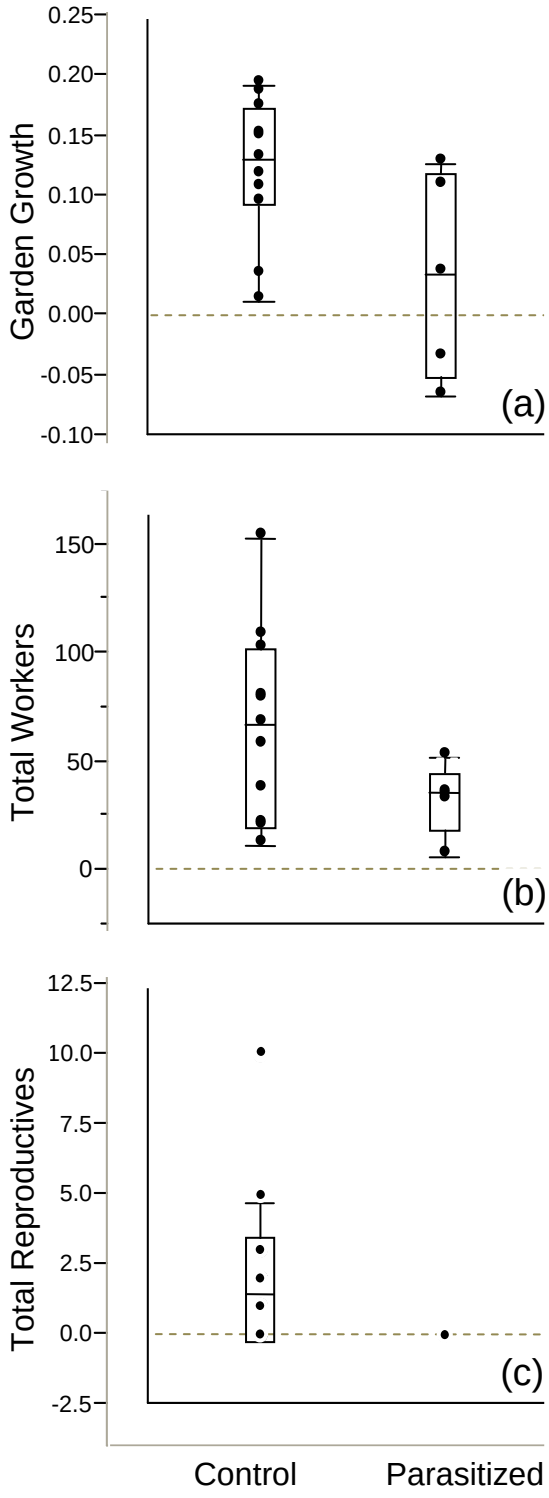

Figure 2.3: a) Average garden weight change ratio (garden growth) for Control and Parasitized colonies. Control garden ratios were significantly larger than the Parasitized colonies ($P = 0.014$). b) The total workers produced in one year in Control and Parasitized colonies were marginally significantly different ($P = 0.05445$). c) The number of total reproductives produced was marginally significantly different between the Control and Parasitized colonies after one year and seven months in the laboratory ($P = 0.0713$) suggesting that there is a negative fitness impact on parasitized host colonies.

Reproductives Produced (1yr 7 months)

After one year and seven months, six Control colonies (n = 10) and none of the Parasitized colonies (n = 4) produced reproductives (sample sizes reduced due to mortality). The total number of reproductives was marginally significantly different (Wilcoxon rank-sum test: Z = -1.8035, P = 0.0713; Control score mean = 8.7 and Parasitized score mean = 4.5).

DISCUSSION

The result of this year-long experiment indicates that the presence of *M. symmetochus* reduces the fitness of its host *T.*cf. *zeteki*; *M. symmetochus* therefore has been appropriately classified as a social parasite. Specifically, two measures of host fitness, garden mass and worker number, decreased when host colonies were parasitized (2.2a, b & Figure 2.3a, b). After a year and seven months, reproductives were found in 60% of the remaining unparasitized Control colonies (n = 10) and in none of the Parasitized colonies (n = 4) (P = 0.0713) (Figure 2.3c). These results support the conclusion that the parasite impacts host fitness negatively, possibly through consumption of fungus garden as well as host larvae.

During artificial introductions the parasite queen experienced aggression from the host queen but not the host workers. Although capable, the parasite queen did not kill the host queen unless continuously rejected by the host. Once fully integrated, the host/parasite association appears amiable, with the host workers grooming the parasite queen, as well as placing bits of fungus on her thorax which may help further disguise the parasite from the host. After parasite infiltration, the host and parasite queens comingle in the nest matrix until the parasite builds her fungal nest cavity, where she generally remains with her brood. Parasite workers rarely initiate contact with the host, with the exception of the female reproductives whom they attack by chewing off their wings (Figure 2.1). This

attack not only impacts the fitness of the host ant colony but also the fitness of the fungal cultivar, which relies on female reproductives for dispersal.

The processes and consequences of infiltration by a social parasite into a well-guarded host society are different for various social parasites. Colony members distinguish nestmates from foreigners by using cuticular hydrocarbons, which can be produced by the ants or acquired from the environment (Singer 1998; Lenoir *et al.* 1999). Hymenopteran social parasites (ants, bees and wasps) have converged on infiltration strategies that exploit the host's recognition system to gain access to colonies (Fisher 1987; Sick *et al.* 1994; Lorenzi *et al.* 2004; Lambardi *et al.* 2007). They accomplish this by mimicking or lacking chemical cues mediating nestmate recognition or using weaponry that alters host behavior (Lenoir *et al.* 2001). The parasite avoids being killed while preserving the host (queen and/or workers) for exploitation. Given the intricate communication system shared by host and parasite, there may only be a small window of opportunity to experimentally introduce parasites into a host colony. For this reason it is not surprising that no one, to our knowledge, has measured the fitness cost to the host in any other cohabiting host/parasite system.

Field surveys have shown that the presence of a social parasite alters social properties of the host. For example, hosts of the inquilines *Solenopsis daguerrei* and *Plagiolepis xene* are typically polygynous but have fewer queens when parasitized (Calcaterra *et al.* 1999; Passera *et al.* 2001). The production of reproductives is slightly delayed in *Solenopsis richteri* when it is parasitized by *Solenopsis daguerrei* (Calcaterra *et al.* 1999). The host is unlikely to be negatively impacted in *Plagiolepis pygmaea*, when it is parasitized by *Plagiolepis xene* inquline (Passera *et al.* 2001). In this latter example, parasite fitness is closely tied to host reproductive output. The two species bud from the natal colony and disperse when the host colony produces reproductives. Consequently, social parasites can impact hosts differently and measuring host fitness in these complex systems can be

multifaceted. Certainly, the number of queens a colony currently has or will produce is important for colony success but other factors that indirectly impact fitness such as worker number are of equal importance. Furthermore, it is ideal to study fitness impacts experimentally by introducing parasites to randomly chosen host colonies of the same age.

Changes in the reproductive output (i.e., males and future queens) of the host colony may be due to a number of factors. The host queen may curtail her reproductive effort in response to a smaller garden (i.e., food store). She may invest in building a work force to tend the garden, and then produce reproductives when the colony reaches an optimal size. Alternatively, the parasite might directly control the number of reproductives eclosing via brood consumption.

According to Forbes (1993) there are three types of parasites, those that dramatically reduce host resources used in current reproduction but impact future reproduction much less (Type I), those that have little influence on current reproduction of the host and greatly reduce future reproduction (Type II), and those that dramatically reduce host resources for current and future reproduction and persist across seasons (Type III). *M. symmetochus* ants are iteroparous like their host, therefore it is likely that they will decrease host fitness throughout the host's lifetime. It seems probable that the parasite would not kill the host colony before it has produced adequate resources for the parasite to maximize lifetime reproduction.

In non-experimental colonies, we observed a unique form of host-castration by the parasite workers. Castration in animals by parasites involves the destruction of gonadal tissue or physiological manipulation of gametogensis or mating behavior (Baudoin 1975). Castration of host queens is found in certain types of social parasites such as slavemaking ants and temporary and permanent social parasites (Wheeler 1910). Social parasites can either suppress reproduction or kill the host queen, using the host colony workers as a

'resource boost' to start and/or maintain their own colony (Hölldobler & Wilson 1990). This is not the case for *M. symmetochus* parasites. They castrate potential host-queens before dispersal thus converting 'host reproductive effort' into energy available for colony maintenance. In other words, these future queens do not disperse, mate, and start their own colony; rather they stay in the colony and perform 'worker' tasks such as foraging (RMM Adams pers. obs.). This shift in behavior presumably improves the host's survivorship, which in turn benefits the parasite.

M. symmetochus fits the Type III parasite category, where the parasite persists across seasons reducing current and future host reproduction. Our results show that colony growth and current reproduction is negatively impacted in the first two years of the host colony life. This fitness depression of young colonies should also impact host colony fitness at older ages. Although older parasitized laboratory colonies produce male and female reproductives, only the host males would be allowed to disperse if they were in the wild (due to female castration; Figure 2.1). Thus, we hypothesize that male reproductives will disperse in nature from mature parasitized host colonies and wing-clipped females will remain in the nest. Our results further show, that in the first year, the parasite colony is just beginning to build a work force and produce reproductives, growing larger with the host colony but beginning reproductive investment earlier. The size of the parasite colonies at the end of our study suggests that more seasons are needed to maximize parasite reproductive output, further showing that the host and parasite cohabit across seasons. Future research, tracking the lifecycle of parasite/host colonies would elucidate lifetime fitness impact of the host and the lifetime reproductive output of the parasite.

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SUPPLEMENTARY MATERIAL

Appendix S2.1: Garden replacement

During the first three months in captivity, most colonies experienced a complete garden loss at least once. The cause of the garden loss was not determined although in some cases the garden appeared to suffer from a bacterial or fungal pathogen. In nature, gardens are susceptible to pathogens and garden loss most likely results in colony death. In the laboratory, the colonies were kept alive by replacing the lost garden with a new garden fragment of the same weight as the original garden fragment. Replacement gardens were taken from the same colony used to supply garden fragments for all experimental colonies (RMMA050105-29). Nest boxes were also replaced, to decrease the likelihood of re-infections. Garden replacements were occasionally necessary during the initial data collection period but much less frequently once colonies were stabilized. A non-parametric independent groups test revealed no significant differences in garden replacements in Control and the Parasitized groups (Wilcoxon rank-sum test: Z = -0.00, P = 1, Control: score mean=8.45, n = 11 and Parasitized: score mean=8.6, n = 5).

Appendix S2.2: Garden mass and worker number status at the start of data collection

Original garden weight

At the start of the data collection period, there was no significant difference in garden weight between Control (n = 11) and Parasitized (n = 5) treatments (t-test: DF 14 = 0.271, P = 0.7909, Control: mean = 328.02mg and Parasitized: mean = 309.32mg; Wilcoxon rank-sum test: Z = -0.22658, P = 0.8208, Control: score mean=8.72727 and Parasitized: score mean=8.0; Levene's Test: P = 0.6892).

Original worker number

At the start of the data collection period, there was no significant difference in worker number between Control (n = 11) and Parasitized (n = 5) groups (Wilcoxon rank-sum test: Z = 0.30051, P = 0.7638, Control: score mean=8.22727 and Parasitized: score mean=9.1).

Table S2.1: Statistical tests allowing the combining of the original control categories (Complement Control (CC) and *Megalomyrmex* Minus (M-) colonies.

By the end of the experiment, there was a severe reduction in sample sizes due to mortality. Because CC and M- were both controls, we tested to see if we could combine the two treatments rather than eliminating the M- data. Because the variances are equal according to the Levene's test, the parametric t-test was used. In addition, a non-parametric Wilcoxon rank-sum test was also used due to the small sample size. The p-values indicate that there were no statistical differences between the two control categories.

	Sample Size		**Two-tail *t*-test**	**Tests of unequal variances: Levene's Test**	**Wilcoxon rank-sum test**
	CC	***M-***			
Garden Weight Change	8	3	P = 0.5477	P = 0.1294	P = 0.6065
Total Workers Produced	8	3	P = 0.6894	P = 0.1344	P = 0.7595
Live Workers at Experiment End	8	3	P = 0.8838	P = 0.3724	P = 1
Reproductives (1yr, 7months)	7	3	P = 0.7035	P = 0.2316	P = 1
Garden Replacements	8	3	P = 0.91	P = 0.2038	P = 0.8219
Garden Weight at Start	8	3	P = 0.5054	P = 0.2713	P = 0.4750
Worker Number at Start	8	3	P = 0.6094	P = 0.9751	P = 0.5726

Table S2.2 Parametric and non-parametric analysis results and tests for unequal variances. Both parametric and nonparametric tests were used due to small sample sizes. Levene's test confirms equal variances.

	Sample Size		One-tail *t*-test	Tests of unequal variances: Levene's Test	Wilcoxon rank-sum test
	Control	***Parasitized***			
Garden Weight Change	11	5	$P = 0.014$	$P = 0.2478$	$P = 0.0616$
Total Workers Produced	11	5	$P = 0.05445$	$P = 0.0606$	$P = 0.1127$
Live Workers at the then End	11	5	$P = 0.05475$	$P = 0.2130$	$P = 0.0999$

Chapter 3:

Venom alkaloids corroborate cryptic species in *Megalomyrmex mondabora*

ABSTRACT

Venom alkaloids were analyzed in the social-parasitic ant *Megalomyrmex mondabora* (Solenopsidini, Formicidae). Samples from Costa Rica contain *trans*-2,-5-dialkylpyrrolidines **1** and **2** and *trans*-2-hexyl-5-[8-oxononyl]-pyrrolidine (**3**). Three samples from Panama contained *trans*-2-methyl-6-alkyl piperidines **4** and **5**. The structures **1**, **2**, **4**, and **5** were all established by direct comparison with authentic samples in the appropriate cis-trans conformation. An authentic sample of *cis*- and *trans*-2-hexyl-5-[8-oxononyl]-pyrrolidine (**3**) was prepared. The piperidines **4** and **5** are known venom components from *Solenopsis* ants, but this is the first report of their occurrence in *Megalomyrmex* ants. Although a ketone-containing indolizidine has been reported in venom alkaloids from the genus *Myrmicaria*, pyrolidine **3** is the first ketopyrrolidine to be observed in *Megalomyrmex* venom. The chemotaxonomic information of the five venom alkaloids corroborates information from phylogenetic, morphological, and natural-history analyses that previously and independently suggested the existence of at least two cryptic ant species within *Megalomyrmex mondabora sensu lato.*

INTRODUCTION

Venom alkaloids found in some ant species in the tribe Solenopsidini function as repellents (Blum *et al.* 1980; Adams & Traniello 1981; Jones *et al.* 1990; Andersen *et al.* 1991), insecticides (Bacos *et al.* 1988), or antiseptic secretions (Jouvenaz *et al.* 1972). In the solenopsidine ant genera *Monomorium*, *Solenopsis*, and *Megalomyrmex*, venom includes a diversity of monocyclic and bicyclic alkaloids (Jones *et al.* 1982a), but also species-specific heterocyclic compounds that can be used as taxonomic characters (Brand 1978; Jones *et al.* 1988). Chemotaxonomy has helped decipher species differences (Brand 1972; Vander Meer 1986), population differences (Vander Meer & Lofgren 1988), and hybridization patterns (Vander Meer & Lofgren 1989) in taxonomically challenging species-complexes, and chemotaxonomic characters can be highly concordant with genetic and morphological characters (Ross *et al.* 1987).

The genus *Megalomyrmex* is comprised of 31 species found in tropical rain forests. The genus as a whole ranges from southern Mexico to Argentina (Brandão 1990, 2003). Most species are free-living predators, whereas a small number of *Megalomyrmex* species are social parasites of fungus-growing ant species. The fungus-growing (attine) ants (Formicidae, Attini) are among textbook examples of co-evolution and mutualism. Their mutualists include their fungal cultivars (Leucocoprineae and Pterulaceae) and actinomycete bacteria that reside on the ants' cuticle (Currie *et al.* 1999; Mueller *et al.* 2005). The tribe Attini contains over 230 described species, divided into the phylogenetically derived "higher attines" (which includes the leaf-cutting ant genera) and the phylogentically basal "lower attines" (Mueller 2002; Mueller *et al.* 2005; Schultz & Brady 2008; Introduction Chapter). The tribe Attini can be further subdivided by distinct fungicultural habits, depending on the type of fungus cultivated by a respective ant lineage. There are five main groupings in ant fungiculture termed 1) lower agriculture, 2) coral fungus agriculture, 3) yeast agriculture, 4) generalized higher agriculture, and 5) leaf-cutter agriculture (Schultz & Brady 2008). In a few cases, ant species from the same

genus may practice more than one form of agriculture.

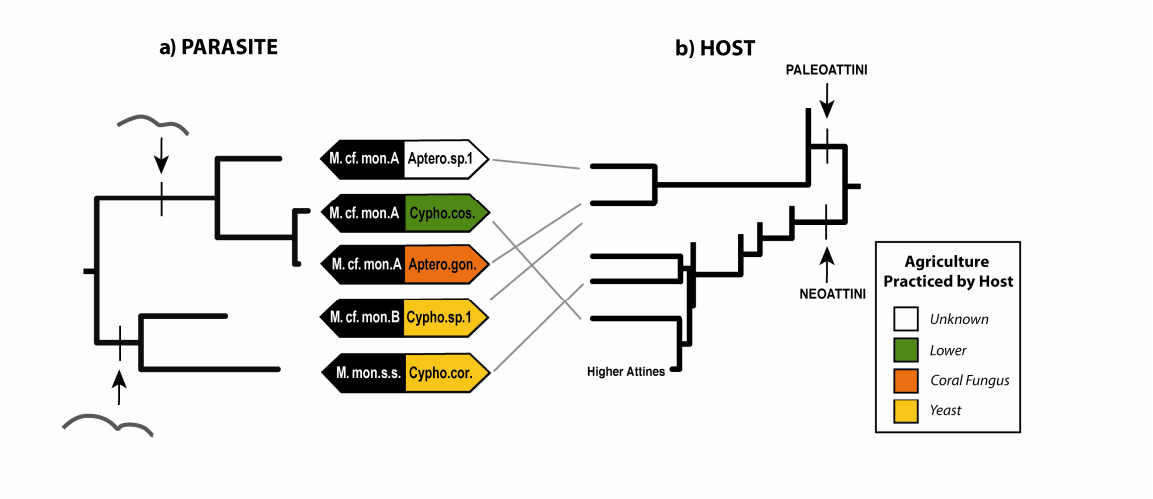


Figure 3.1: Parasite and host phylogenies. a) This phylogeny is the *M. mondabora s.l.* clade from the Bayesian *Megalomyrmex* phylogeny in Chapter 4. The line drawing is a depiction of the ant size and the metanotal groove (the dorsal side of the thorax viewed from the side) which are morphological characters that differentiates *M.* cf. *mondabora* A clade from *M. mondabora s.s.* and *M.* cf. *mondabora* B clade. b) Fungus-growing ant phylogeny (adapted from Schultz & Brady 2008). Only the clades that contain the taxa that are *M. mondabora s.l.* hosts are included. Fungiculture practices are indicated by the background coloration of the host labels (see key). The fungicultural practice of *Apterostigma* sp. 1 is unknown. Paleoattini and Neoattini clades are indicated by arrows. Aptero. sp. 1 = *Apterostigma* sp. 1; Cypho. cos. = *Cyphomyrmex costatus*; Aptero. gon. = *Apterostigma goniodes*; Cypho. sp 1 = *Cyphomyrmex* sp. 1; Cypho. cor. = *Cyphomyrmex cornutus*.

Megalomyrmex mondabora sensu lato, *Megalomyrmex symmetochus*, and *Megalomyrmex wettereri* associate with several attine species, some spanning the attine phylogeny (except the leaf-cutting ant species) and are not known to exist in a non-parasitic, predatory condition unassociated with attine ants (Brandão 2003; Adams & Dewitz 2006; Adams & Longino 2007; Introduction Chapter). *Megalomyrmex mondabora s.l.* and *M. symmetochus* are social parasites living inside their host nest or in close proximity (e.g., nest walls of host) (Adams & Longino 2007; Chapter 2). In contrast, *M. wettereri* is either a social parasite or an agro-predator that usurps the host nest, depending on the attine host species (Adams *et al.* 2000). All three species consume

the host fungal garden and brood. In spite of the remarkable diversity of cultivar types, the associated attine species remain susceptible to *Megalomyrmex* parasites (Figure 3.1b).

The ant *Megalomyrmex mondabora s.l.*, as defined by current taxonomic criteria, associates with seven different fungus-growing ant species (Adams & Longino 2007; Table 3.2). All hosts are -considered lower-attine ants but they practice an array of fungus agriculture. For example, *Cyphomyrmex costatus* cultivates a fungus that belongs to the tribe Leucocoprinae (parasol mushrooms), *Apterostigma goniodes* cultivates coral fungi (Pterulaceae), and *Cyphomyrmex cornutus* and *Cyphomyrmex* sp. 1 are yeast-growing ants that cultivate highly derived leucocoprinaceous fungi that grow as a yeast (single-celled) rather than as a filamentous (hyphal) fungus when associated with ants.

Megalomyrmex mondabora s.l ants show variation in size and morphological detail (J. T. Longino personal communication; Figure 3.1a). This morphological variation (e.g., shape of the propodeal lobe, degree of impression of metanotal groove, color), in conjunction with the numerous host species, prompted us to suspect in a previous report that *M. mondabora s.l.* may actually encompass multiple cryptic species (Adams and Longino 2007). In the present report, we 1) summarize the natural history of *M. mondabora s.l.*, specifically addressing how the diverse host affiliations relate to the parasite's taxonomy; 2) discuss the phylogenetic relationships and molecular divergences between samples of *M. mondabora s.l.* affiliated with different host species; and 3) describe the venom alkaloids of *M. mondabora s.l.*, including a novel compound never before found in *Megalomyrmex*. The results support the conclusion that *M. mondabora* is a species complex with at least two cryptic species.

MATERIALS AND METHODS

Ants

Megalomyrmex mondabora s.l. samples were collected between 1999 and 2006 in Costa Rica, Ecuador, Panama, and Peru (see Table 3.2 for collection information). Three to ten whole ants were placed in vials containing 100% methanol for chemical analysis. Voucher specimens are deposited at the entomological collections of the National Museum of Natural History of the Smithsonian Institution (Washington, DC) and at The Evergreen State College (Olympia, Washington).

Chemical Analyses

GC-MS analyses of venom alkaloids was carried out in the EI mode using a Shimadzu QP-5000 GC-MS equipped with an RTX-5, 30 m x 0.25 mm i.d. column. The instrument was programmed from 60 ^{o}C to 250 ^{o}C at 10 ^{o}/min. Vapor phase FT-IR spectra were obtained using a Hewlett-Packard model 5965B detector interfaced with a Hewlett-Packard 5890 gas chromatograph fitted with a 30 m x 0.25 mm RTX-5 amine column. NMR spectroscopy was carried out in $CDCl_3$ solutions using a Varian Mercury 400 NMR spectrometer. HRMS was performed on a JEOL SX102 instrument in the positive-ion fast-atom bombardment mode using a direct probe and a Waters LCT Premier Time of Flight instrument in the electrospray (ESI) mode.

Alkaloid identification

The identity of the alkaloids from *M.* cf. *mondabora* A were established by direct comparison with authentic samples.

The methanol extracts of *M. mondabora s.s.* showed the presence of three alkaloids in a 32:1:21 ratio. The first and second of these had mass spectra and gas chromatographic retention times identical to those of authentic samples of trans-2-heptyl-5-

hexylpyrrolidine and trans-2-hexyl-5-nonylpyrrolidine (Jones *et al.* 1982b; Jones *et al.* 1991). The third compound had an EIMS *m/z* 294 [M-1$^+$] (1), 238 (2), 236(2), 210 (71), 194 (10), 180 (5), 154 (100), 82 (32), 69 (21), 43 (36), and an absorption at 1731 cm^{-1} in its GC-FTIR spectrum. Treatment of the mixture with a small amount of $NaBH_4$, followed by acidification, neutralization and ether extraction changed the mass spectrum of the last eluting alkaloid to EIMS *m/z* 296 [M-1$^+$] (1), 282 (2), 212 (20), 194 (12), 180 (1), 154 (100), 82 (16), 69 (28), 55 (26), 45 (36), 43 (36), 41 (42).

Ethyl 2-acetyl-9-hydroxynonanoate (6)

Freshly distilled ethyl acetoacetate (4.4 ml, 35 mmol) was added dropwise to 30 ml of EtOH in which 0.8 g of sodium had been dissolved, followed by the addition of a solution of 4.9 g (25 mmol) of 7-bromoheptanol (Kang *et al.* 1985) in 20 ml of EtOH. The mixture was heated to reflux overnight under a drying tube. Upon cooling, the mixture was neutralized with 10% HCl, and the solvent was removed *in vacuo*. The residue was partitioned between ether and water and the ether extracts were dried over anhydrous $MgSO_4$, filtered, and the solvent was removed to provide 5 g of **6** that was >85% pure and suitable for the next step. Small amounts of **6** were purified by kugelrohr distillation at 0.1 mm Hg. ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.15 (2H, q, *J* = 7.2 Hz), 3.58 (2H, t, J = 7.0 Hz), 3.36 (1H, t, J = 7.6 Hz), 2.18 (3H, s), 1.9-1.75 (3H, complex m), 1.50(2H, m) 1.27 (8H, complex m) 1.32 (3H, t, J = 7.2 Hz); EIMS *m/z* 226 [M$^+$-18] (0.5), 172 (1), 143 (15), 138 (19), 130 (70), 101 (40), 88 (13), 84 (22), 73 (40), 55 (30), 43 (100). HRMS *m/z* 226.1554 ([M-H_2O]$^+$), calcd. for $C_{13}H_{22}O_3$, 226.1569.

2-Methyl-2-[8-oxooctyl]-1,3-dioxolane (7)

A mixture containing 4.8 g (19.6 mmol) of **6** and 1.5 g of H_3BO_3 was stirred and heated in an oil bath at 170 ^{o}C for 2 hr. Upon cooling, the mixture was taken up in ether and carefully neutralized with $NaHCO_3$. The ether extracts were dried over anhydrous $MgSO_4$, filtered, and the solvent was removed to provide 4 g of crude (80% pure) 10-

hydroxy-2-decanone EIMS *m/z* 172 [M^+] (0.5), 97 (10), 96 (15), 71 (28), 58 (100), 55 (40), 43 (95). This product was dissolved in 100 ml of benzene containing 5 ml of ethylene glycol and 0.2 g of p-toluenesulfonic acid and the mixture was heated under reflux under a Dean-Stark trap until all separation of water ceased. The mixture was cooled, washed with saturated $NaHCO_3$, dried over $MgSO_4$, and after filtration, the solvent was removed to provide 2-methyl-2-[8-hydroxyoctyl]-1,3-dioxolane nearly quantitatively, ^{13}C NMR (100 MHz, $CDCl_3$) δ 110.45, 64.80 (2C), 63.09, 39.38, 32.92, 29.99, 29.57, 25.86, 25.81, 24.19, 23.91; EIMS *m/z* 215 [M-1^+] (0.5), 201 [M-15^+] (12), 87 (100), 69 (5), 55 (10), 43 (33). A solution containing 1.3 g (6 mmol) of 2-methyl-2-[8-hydroxyoctyl]-1,3-dioxolane in 3 ml of CH_2Cl_2 was added to a mechanically stirred mixture of 2 g of pyridinium chlorochromate and 0.5 g of NaOAc in 20 ml of CH_2Cl_2. After two hours the usual work up (Corey & Suggs 1975) provided 0.8 g of **7** that was ca. 80% pure by GC-MS. Small amounts of **9** were purified by kugelrohr distillation at 0.1 mm Hg. ^1H NMR (400 MHz, $CDCl_3$) δ 9.73 (1H, t, J = 1.6 Hz), 3.90 (4H, m), 2.31 (2H, t, J = 7.2 Hz), 1.59 (4H, m), 1.35-1.25 (11H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 179.93, 110.42, 64.79 (2C), 39.34, 34.23, 29.80, 29.38, 29.15, 24.85, 24.19, 23.90; EIMS *m/z* 213 [M-1^+] (0.5), 199 [M-15^+] (11), 87 (100), 67 (3), 55 (8), 43 (28); HRMS *m/z* 199.1234 ([M-CH_3]$^+$), calcd. for $C_{11}H_{19}O_3$, 199.1334.

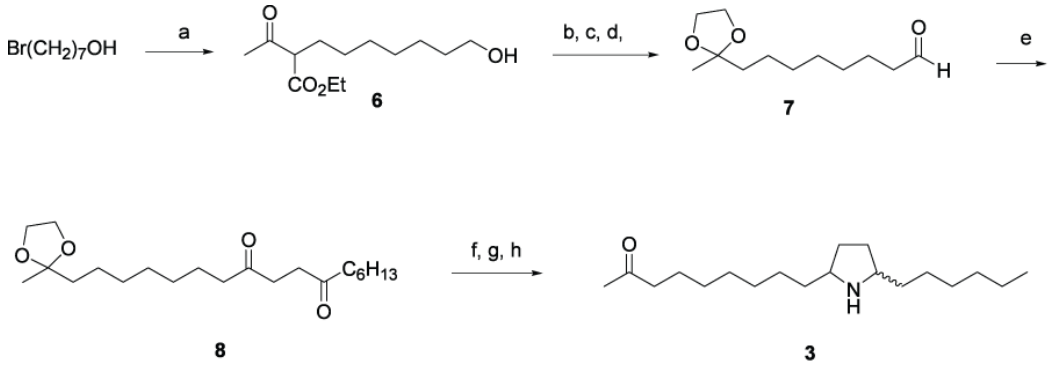
2-[8, 11-dioxoheptadecyl]-2-methyldioxolane (**8**)

A mixture of 1.35 g (9.6 mmol) of 1-nonen-3-one (Jones *et al.* 1991), 1.76 g of **9** , and 0.6 g of 5-(2-hydroxyethyl)-4-methyl-3-benzylthiazolium chloride was treated with 3 ml of freshly distilled triethylamine and refluxed overnight under an argon atmosphere. The mixture was cooled, diluted with ether and filtered and the solvent removed *in vacuo*. Kugelrohr distillation (100-150 at 0.1 mm Hg) provided 2.0 g of **8**. ^{13}C NMR (100 MHz, $CDCl_3$) δ 208.90, 208.85, 109.15, 63.58 (2C), 41.87, 41.83, 38.15, 34.98 (2C), 30.56, 28.64, 28.32, 28.09, 27.85, 23.02, 22.78(2C), 22.69, 21.47, 13.02; EIMS *m/z* 354 [M^+] (1), 339 [M-15^+] (10), 293 (5), 269 (2), 225 (1), 223 (2), 213 (2), 197 (2) 184 (1), 169 (2),

157 (5), 115 (6), 99 (5), 87 (100), 43 (35); HRMS *m/z* 339.2499 ($[M\text{-}CH_3]^+$), calcd. for $C_{20}H_{35}O_4$, 339.2468.

2-Hexyl-5-[8-oxononyl]-pyrrolidine (**3**)

A solution containing 0.2 g (0.57 mmol) of **8**, 0.1 g of NH_4OAc, 0.1 g (2.7 mmol) $NaCNBH_3$, and 2 drops of 10% NaOH in 5 ml of methanol was stirred overnight at room temperature. The solvent was removed *in vacuo* and a small sample was partitioned between water and ether. GC-MS analysis of this aliquot revealed two major components with identical mass spectra corresponding to both isomers of the ethylene ketal of **3**. EIMS *m/z* 338 [M-1$^+$] (1), 324 (7), 254 (46), 224 (8), 210 (21), 154 (100), 87 (93), 82 (18), 43 (35); HRMS *m/z* 324.2935 ($[M\text{-}CH_3]^+$), calcd. for $C_{20}H_{38}NO_2$, 324.2903. The remainder of the reaction mixture was quickly acidified with 10% HCl, and after 10 min neutralized with solid $NaHCO_3$, and extracted with ether (2 x 10 ml). The ether was dried over $MgSO_4$, filtered and the solvent removed *in vacuo* to provide 0.15 g of a 1:1 mixture of the stereoisomers of pyrrolidine **3** that was 71% pure by GC-MS, whose GC retention times were 23.52 min, GC-FTIR ν_{max} 2933, 2866, 1731 (s), 1459, 1360, 1160 cm^{-1}; and 23.61 min, GC-FTIR ν_{max} 2933, 2866, 1731 (s), 1460, 1362, 1119 cm^{-1}; respectively, and which exhibited identical mass spectra. EIMS *m/z* 294 [M-1$^+$] (1), 238



Scheme 3.1: Synthesis of 2-hexyl-5-[8-oxononyl]-pyrrolidine **3**. Reagents: (a) Sodium ethanolate, ethylacetoacetate. (b) H_3BO_3, 170 ^oC, (c) $(CH_2OH)_2$, H^+, (d) PCC/ NaOAc, (e) 1-Nonen-3-one, thiazolium salt, Et_3N, (f) $NaCNBH_3$, NH_4OAc, (g) H^+, (h) $NaHCO_3$

(2), 236 (2), 210 (71), 194 (10), 180 (5), 154 (100), 82 (32), 69 (21), 43 (36); HRMS *m/z* 210.1820 ($[M-C_6H_{13}]^+$), calcd. for $C_{13}H_{24}NO$, 210.1854. The GC-FTIR, EIMS and retention time of the second eluting isomer of **3** were identical to those of the last eluting alkaloid in *M. mondabora s.s.* Treatment of the mixture with a small amount of $NaBH_4$, followed by acidification, neutralization and ether extraction provided a pair of isomeric pyrrolidine alcohols whose mass spectra were identical to that obtained from the $NaBH_4$ of the *M. mondabora s.s.* extract.

Genetic distances

Using sequencing information gathered for Chapter 4, the pairwise uncorrected 'p' genetic distances were estimated in PAUP* (Swofford 2001) for five *M. mondabora s.l.* taxa. These taxa formed a monophyletic group in phylogenetic analyses presented in Chapter 4 and represent the known diversity of *M. mondabora s.l.* types. In ants, a 2-3% sequence divergence threshold is generally accepted for testing species delineation hypotheses (Smith et al. 2005a; Smith et al. 2005b). Even though distance methods are not a fail-safe method to delineate valid taxonomic lineages (Ferguson 2002), they can serve as corroborative evidence to support the conclusions of other taxonomic methods.

Taxonomic work

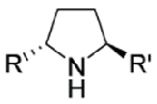
Without knowledge of the phylogenetic and chemical results presented in this paper, (Figure 3.1; Chapter 4) an independent morphological study was conducted in order to identify character-state differences, if any, that separated *M. mondabora s.l.* into two or more species. Three morphometric characters were used: head length, head width, and scape length (data not shown, see: http://academic.evergreen.edu/projects/ants/GENERA/MEGALOMYRMEX/SPECIES/mondabora/mondabora.html). Other morphological characters were the shape of the dorsal margin of the posterior propodeal lobe, depth of the metanotal groove (Figure 3.1a), and color. Nineteen collections were examined and categorized into three groups based on differences in these characters: *M. mondabora*

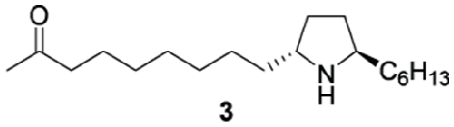
sensu stricto, *M.* cf. *mondabora* A, and *M.* cf. *mondabora* B (Table 3.2). An Automontage image (three-dimensional picture) of the holotype was referenced to determine which samples best represent *M. mondabora s.s.* (not included in Table 3.2).

RESULTS AND DISCUSSION

The structures of the *trans*-2,-5-dialkylpyrrolidines **1** and **2** and of the *trans*-2-methyl-6-alkyl piperidines **4** and **5** found in the *M. mondabora s.l.* samples were all established by direct comparison with authentic samples available from previous studies (Table 3.1) (Jones *et al.* 1982b; Jones *et al.* 1989).

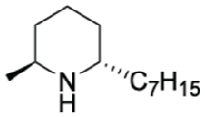
In *M. mondabora s.s.*, a compound with important fragments in its EIMS at *m/z* 294 [M-1^+] (1), 238 (2), 236 (2), 210 (71), and 154 (100), and having a strong absorption at ν_{OH} 1731 cm^{-1} was detected along with equivalent amounts of **1** and a trace of **2**. Sodium borohydride reduction of a small portion of this mixture converted this carbonyl containing component into a compound whose EIMS had significant fragments at *m/z* 296 [M-1^+] (1), 282 (2), 212 (20), 194 (12), 180 (1), 154 (100), and 45 (36). These data suggested a ketone at the penultimate carbon of the C-9 carbon chain whose reduction provided an alcohol showing a loss of CH_3 (*m/z* 282) and a CH_3CHOH fragment at (*m/z* 45). An authentic sample of *cis*- and *trans*-2-hexyl-5-[8-oxononyl]-pyrrolidine (**3**) was prepared (Scheme 3.1), and the natural pyrrolidine had identical GC-FTIR, mass spectrum, and retention time as the second eluting isomer. In 2,5-disubstituted pyrrolidines, the *trans* isomer is the second eluting isomer, and does not have a shoulder on the lower frequency side of the Bohlman region in the GC-FTIR that is observable for the cis isomer (Garraffo *et al.* 1994).





3

1 R = C_7H_{15} R' = C_6H_{13}
2 R = C_9H_{19} R' = C_6H_{13}



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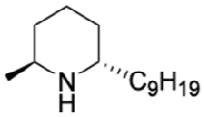


5

Species **Collection number** **Collection site**	**Alkaloids**				
	1	**2**	**3**	**4**	**5**
M. mondabora s.s. RMMA030213-09 El Ceibo, CR	++	o	++		
M. mondabora s.s. RMMA030213-07 El Ceibo, CR	++	o	++		
M. mondabora s.s. RMMA050625-01 El Ceibo, CR	++	o	++		
M. cf. *mondabora* A HF010330-50 El Llano, PA				+	++

Table 3.1: Venom alkaloids from *Megalomyrmex mondabora s.s. and M. cf. mondabora* A workers; ++ = major component; + = minor component (5-20%); o = trace (< 1%); "empty cell" = not detected.

It is noteworthy that, while the piperidines **4** and **5** found in *M*. cf. *mondabora* A are well known as venom components from *Solenopsis* ants (Jones *et al.* 1982a), this is the first report of their occurrence in a *Megalomyrmex* species. Previously, *Megalomyrmex* species have been characterized by the presence of a five-membered ring containing pyrrolidines and pyrrolizidines. The significance of this observation lies in the different biosynthetic pathways required for piperidines as opposed to pyrrolidines. The polyacetate pathway to the *Solenopsis* piperidines has been established (Leclercq *et al.*

1996), whereas the biosynthesis of simple 2,5-dialkyl pyrrolidines has not been elucidated, although studies of alkaloids from *Tetraponera* are known to require amino-acid as well as acetate precursors (Morgan 2004).

Furthermore, although a ketone-containing indolizidine has been reported in venom alkaloids from the genus *Myrmicaria* (Francke *et al.* 1995), pyrrolidine **3** is the first ketopyrrolidine to be observed in a *Megalomyrmex* venom. The nineteen-carbon 2-hexyl-5-nonylpyrrolidine carbon skeleton is common to North American *Monomorium* species (Jones *et al.* 1982b). In those ants, the side chains often contain terminal alkenes. In the case of **3**, the carbonyl on the penultimate carbon of the nine-carbon side chain may indicate a common biosynthesis except for the establishment of functionality at the end of that side chain.

Species delineation and host choice

Megalomyrmex mondabora, as currently defined, may actually be several cryptic *M. mondabora*-like species that comprise a species complex. Phylogenetic patterns indicate that there are two clades, one containing Panamanian and Peruvian samples (*M.* cf. *mondabora* A) and the other containing Ecuadorian and Costa Rican samples (*M.* cf. *mondabora* B and *M. mondabora s.s.*) (Figure 3.2). Geographic distance between these possible lineages likely contributes to divergence, however because the Peruvian and Panamanian taxa form one clade and the Costa Rican and Ecuadorian samples form another, it appears that these two clades overlap geographically across a large area. Furthermore, taxa belonging to different morphological categories were found sympatrically (see below) suggesting that other biological factors, besides geography, are responsible for the divergence observed. Morphological characters and genetic distances suggest that there are at least two distinct lineages (but see below).

Megalomyrmex mondabora s.s.

All of the *M. mondabora s.s*. samples examined were found in Costa Rica and Panama, with the exception of the Paratype from Vilhena, Brazil (Table 3.2) (Brandão 1990). However, the paratype was classified as *M*. cf. *mondabora* A in this study, indicating that the variation in morphology observed here was considered when *M. mondabora* was originally described (Brandão 1990). *Megalomyrmex mondabora s.s.* parasitize three host species, *Apterostigma* cf. *goniodes* (a coral-fungus agriculturist) and two yeast growing species, *Cyphomyrmex cornutus* and *Cyphomyrmex salvini*. The latter two host species were collected in sympatry, indicating that a single *M. mondabora s.s.* population may associate with more than one host species.

Megalomyrmex cf. mondabora A

Megalomyrmex cf. *mondabora* A samples were collected from Panama, Ecuador, Brazil, and Peru (Table 3.2). They associate with five different host species that practice three different types of agriculture (lower, coral-fungus, and yeast agriculture) (see Figure 3.1b; Note: *Cyphomyrmex* sp. 1 is not represented). Two hosts are mycelium-growing attines, *Cyphomyrmex costatus* (a lower agriculturist) and *Apterostigma* goniodes (a coral-fungus agriculturalist), whereas *Cyphomyrmex* sp. 1 is a yeast-growing attine (Table 3.2). The agricultural systems of *Apterostigma* sp. 1 and *Apterostigma* sp. 2 are unknown, except that they are mycelium cultivators.

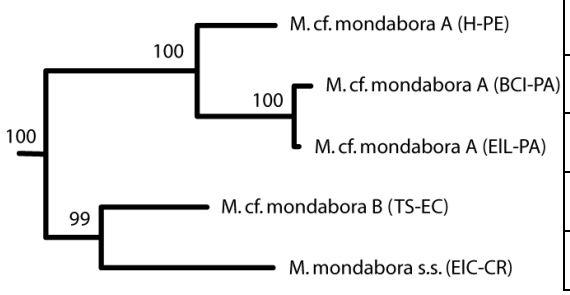
Megalomyrmex cf. mondabora B

Only one sample of *M*. cf. *mondabora* B was examined. Interestingly, it occurred sympatrically in Ecuador with *M*. cf. *mondabora* A and was collected within a colony of a host species (yeast-growing *Cyphomyrmex* sp. 1) also parasitized by *M*. cf. *mondabora* A (Figure 3.1; Table 3.2). *M* cf. *mondabora* A also occurs sympatrically with *M. mondabora s. s*. in Panama, both parasitizing *Apterostigma goniodes*.

Genetic distances and morphology

Megalomyrmex cf. *mondabora* A from two sites in Panama (EL-PA & BCI-PA), Ecuador (TS-EC) and Peru (H-PE) share several morphological characters (comparable size, dorsal margin of posterior propodeal lobe somewhat angular, and a moderately impressed metanotal groove) (Figure 3.1a). These samples were collected with *A. goniodes* and *C. costatus*, *C.* sp. 1, and *A.* sp. 1, respectively (Table 3.2). The two Panamanian samples showed a minimal genetic distance of 0.9% (Figure 3.2). One sample was collected near El Llano; the other sample was collected about 100 km away on Barro Colorado Island. The Peruvian sample's genetic distance is 5.9% from the BCI-PA sample and 5.4% from the EL-PA sample. *Megalomyrmex mondabora s.s.*, a parasite of *Cyphomyrmex cornutus* from Costa Rica, has the greatest genetic distance from all other samples, 10%, 10.2%, and 9.9% from all three *M.* cf. *mondabora* A samples (H-PE, BCI-PA, EL-PA, respectively). Finally, *M. mondabora s.s.* and *M.* cf. *mondabora* B share several

Figure 3.2: Genetic distances between *M. mondabora s.l.* taxa based on the sequences of COI and wingless genes. The phylogeny is the *M. mondabora s.l.* clade subset of the *Megalomyrmex* phylogeny presented in chapter 4. Numbers at the nodes are Bayesian posterior probabilities.



	M. cf. *mondabora* A (H-PE)	*M.* cf. *mondabora* A (BCI-PA)	*M.* cf. *mondabora* A (EIL-PA)	*M.* cf. *mondabora* B (TS-EC)	*M. mondabora* s.s. (EIC-CR)
M. cf. *mondabora* A (H-PE)	*	*	*	*	*
M. cf. *mondabora* A (BCI-PA)	5.9%	*	*	*	*
M. cf. *mondabora* A (EIL-PA)	5.4%	0.9%	*	*	*
M. cf. *mondabora* B (TS-EC)	8.1%	8.7%	8.4%	*	*
M. mondabora s.s. (EIC-CR)	10%	10.2%	9.9%	8.4%	*

morphological characters that make them more similar to each other than to the Panama and Peru samples of *M*. cf. *mondabora* A. The metanotal groove is more incised (Figure 3.1a), the propodeum is more swollen, and the color is more uniformly and darker black. The genetic distance between *M*. cf. *mondabora* B (TS-EC) and three *M*. cf. *mondabora* A samples are similar (H-PE=8.1%, BCI-PA=8.7%, and EL-PA=8.4%), as is the genetic distance between *M. mondabora s.s.* (ElC-CR) and *M*. cf. *mondabora* B (TS-EC) (8.4%) regardless of their shared morphological characters, sister taxa position on the phylogeny, and preference for parasitizing yeast-growing agriculturists (Figure 3.1a). All taxon comparisons besides the two samples collected in Panama are well above the 2-3% sequence divergence threshold, suggesting that there may be as many as four cryptic species (but see Smith et al. 2005a).

Unfortunately, none of the *M. mondabora s.l.* samples that occurred sympatrically with the other *M. mondabora* lineages were sequenced (e.g. *M. mondabora s.s.* and *M*. cf. *mondabora* A from El Llano, Panama; *M*. cf. *mondabora* A and *M*. cf. *mondabora* B from Toachi Station, Ecuador). Therefore genetic distance and phylogenetic positions of these two paired taxa could not be determined in this study.

According to morphological and phylogenetic criteria, there appear to be three to four cryptic species in the *M. mondabora*-complex: The *M*. cf. *mondabora* A (BCI-PA and ElL-PA), *M*. cf. *mondabora* A (H-PE), *M*. cf. *mondabora* B and *M. mondabora s.s.* Further subdivision is possible but less clear at this time. Our final line of evidence that corroborates species delineation is the venom alkaloids, but only two of the four cryptic species were chemically analyzed. *Megalomyrmex mondabora s.s.* (ElL-CR) produce *trans*-dialkylpyrrolidines **1** and **2** and *trans*-2-hexyl-5-[8-oxononyl]-pyrrolidine (**3**) and *M*. cf. *mondabora* A (El-PA) produce *trans*-2-methyl-6-alkyl piperidines **4** and **5** (Table 3.1). The biochemical pathway of pyrrolidines compared to piperidines is striking suggesting species divergence. Until the other cryptic species are chemically analyzed,

we recommend the recognition of a single new species. *M. mondabora s.s.* (ElC-CR) and *M.* cf. *mondabora* A (ElL-PA) are convincingly distinct morphologically, genetically (forming two separate phylogenetic clades and are 10% divergent) and chemically.

To formally describe a new species in this taxonomically challenging species complex, a more extensive α-taxonomic revision is needed, ideally drawing also on information from genetic and chemical analyses. This multifaceted approach to species delineation is recommended to elucidate the cryptic speciation that appears to be occurring in the *M. mondabora s.l.* clade. *M. mondabora s.s.* (ElC-CR) and *M.* cf. *mondabora* A (ElL-PA) occur sympatrically with the same host species but also occur allopatrically, associating with numerous other host species. Analysis of molecular variance and geneflow within and between populations could provide further clues for lineage differentiation (Ferguson 2002). It would also be interesting to determine if sympatric *M. mondabora s.l.* colonies parasitizing different host species are diverged or interbreed freely. In addition, a study on mate preference could elucidate reproductive isolation mechanisms to further test for cryptic species in the *M. mondabora*-complex.

Table 3.2: *Megalomyrmex mondabora s.l.* samples analyzed morphologically, chemically or genetically.

ID number **Collector** **Name**	**Analyzed:** Morphologically (M), Chemically (C), & Genetically (G)	**Collection site information** **(code for phylogeny)** **GPS**	**Host species**
AGH030212-13 **A. G. Himler** ***M. mondabora s.s.***	M, G	El Ceibo Research Station, Costa Rica (ElC-CR) 10.31632 N -54.8283W	*Cyphomyrmex cornutus*
CC010324-50 **C. Currie** ***M. mondabora s.s.***	M, G	El Llano, Panama (ElL-PA) 9.279555N -78.9615W	*Apterostigma* cf. *goniodes*
INBIOCRI001280853 **J. T. Longino** ***M. mondabora s.s.***	M	Guanacaste Conservation Area, Est. Pitilla, Costa Rica 10.98333 °N 85.43333°W, 700m	unknown
JTLC000001521 **J. T. Longino** ***M. mondabora s.s..***	M	Heredia, Costa Rica 22km N Volcan Barba 10°20'N084°04'W, 500m	unknown
JTL000006054 **J. T. Longino** ***M. mondabora s.s.***	M	Prov. Alajuela, Peñas Blancas Valley, Refugio Eladio, Costa Rica 10.31667°N 84.71667°W, 800m	unknown
RMMA030213-07 **R. M. M. Adams** ***M. mondabora s.s.***	M, C	Cascante Refuge, Cost Rica 10°20'N 84°04'W 200, 450-550m	*Cyphomyrmex cornutus*
RMMA030213-09 **R. M. M. Adams** ***M. mondabora s.s.***	M, C	Cascante Refuge, Cost Rica 10°20'N 84°04'W 200, 450-550m	*Cyphomyrmex cornutus*
RMMA030219-03 **R. M. M. Adams** ***M. mondabora s.s.***	M "queen"	Cascante Refuge, Cost Rica 10°20'N 84°04'W 200, 450-550m	*Cyphomyrmex salvini*
RMMA050625-01 **R. M. M. Adams** ***M. mondabora s.s.***	M, C	El Ceibo Research Station, Costa Rica 10.31632 N -54.8283W	*Cyphomyrmex cornutus*
RMMA050627-01 **R. M. M. Adams** **M. *mondabora s.s.***	M	El Ceibo Research Station, Costa Rica 10.31632 N -54.8283W	*Cyphomyrmex cornutus*
CC011025-02 **C. Currie** ***M.* cf. *mondabora* A**	M	Pipeline Road, Panama 9.1600N -79.7449W	*Cyphomyrmex costatus*
CC030614-03 **C. Currie** ***M.* cf. *mondabora* A**	M	Tiputini, Ecuador Lat. 0° -45' 0 N, Lon. 75° 31' 60 W	*Apterostigma* sp. 1
HF010330-50 **H. Fernandez-Marin** ***M.* cf. *mondabora* A**	M, C, G	El Llano, Panama (ElL-PA) 9.279555N -78.9615W	*Apterostigma goniodes*
M. mondabora **Paratypus 1986** **M. Alvarenga** ***M.* cf. *mondabora* A**	M	Vilhena: RO, Brazil XI-1973, 10221	unknown
RMMA010324-01 **R. M. M. Adams** ***M.* cf. *mondabora* A**	M	El Llano, Panama 9.279555N -78.9615W	*Apterostigma* cf. *goniodes*
RMMA040528-08 **R. M. M. Adams** ***M.* cf. *mondabora* A**	M	Huacaria, Peru Lat. -12.9027 Lon. -71.4236, 597m	*Apterostigma* sp. 2
RMMA041223-04 **R. M. M. Adams** ***M.* cf. *mondabora* A**	M, G	Barro Colorado Island, Panama (BCI-PA) 9.1648N -79.8366W	*Cyphomyrmex costatus*

RMMA060315-03 **R. M. M. Adams** ***M*. cf. *mondabora* A**	M	Unión del Toachi Station, Ecuador 00˚79`75.7”S 78˚57`05.8”W 820m	*Cyphomyrmex* sp. 1
RMMA060315-02 **R. M. M. Adams** ***M*. cf. *mondabora* B**	M	Unión del Toachi Station, Ecuador 00˚79`75.7”S 78˚57`05.8”W 820m	*Cyphomyrmex* sp. 1

ACKNOWLEDGEMENTS

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Chapter 4:

Phylogeny of the of the ant genus *Megalomyrmex*: Implications for the evolution of social parasitism

ABSTRACT

Social parasitism, the exploitation of one society by another, is a behavior found in some, but not all, ant species of the genus *Megalomyrmex*. Ants in this genus inhabit tropical rainforest leaf-litter environments, ranging from southern Mexico to Argentina. The genus currently comprises 31 species, classified into four species groups based on taxonomic characters. The *leoninus*, *modestus*, and *pusillus* species groups all contain predatory species that hunt other insects. In the *silvestrii* species group, most species are social parasites of fungus-growing ants (Attini), cohabiting with the host and consuming the fungus and brood, but one species is an agro-predator of fungus-growing ants, usurping the attine nest and fungus garden. Using nucleotide sequence data from the (nuclear) wingless and (mitochondrial) cytochrome oxidase I genes, we test the monophyly of each of the four species groups in the genus *Megalomyrmex,* as defined previously based on morphological characters, then reconstructed the evolution of social parasitism within the genus. We specifically address Darwin's Predation Hypothesis, which states that social parasites evolve from predatory species. We found that the two adequately sampled species groups (*leoninus* and *silvestrii*) were recovered as monophyletic and the Predation Hypothesis was supported.

INTRODUCTION

Social insects are remarkable model organisms in evolutionary biology, due to their complex adaptations and associations. They offer many examples of interesting life history characteristics such as symbiotic relationships. Large colony and nest sizes of social insects serve as predictable resources for diverse commensals, predators, and parasites (Hölldobler & Wilson 1990). Some of these parasites are other social insects, which are known as "social parasites". Social parasitism has evolved multiple times within the social Hymenoptera (ants, bees, and wasps), resulting in an array of convergent strategies used by the social parasites to enter and integrate themselves into host colonies (Fisher 1987; Lambardi et al. 2007; Lorenzi et al. 2004). These strategies give social parasites opportunities for exploitation that likely influences the evolution of host species (Foitzik et al. 2003).

Social parasites in the ant tribe Solenopsidini vary greatly in their natural histories. Most are workerless inquilines, dependent on the host to feed them and care for their brood (Hölldobler & Wilson 1990). Other species are xenobiotic (living integrated within a host colony) or lestobiotic (living in close proximity to a host, as for example in nest walls). Both xenobiotic and lestobiotic species acquire food from the host colony and have a worker caste that cares for their broods which are kept separate from the host brood (e.g., *Megalomyrmex symmetochus*, *Monomorium metoecus*, *Oxyepoecus bruchi*) (Adams unpublished; de Albuquerque & Brandão 2004; Hölldobler & Wilson 1990; Wilson & Brown 1958). Xenobiotic and lestobiotic social parasites can be either facultative or obligate associates (Adams 2004; Hölldobler & Wilson 1990). Lastly, social parasites can parasitize species of the same genus (e.g., *Solenopsis* parasites on *Solenopsis* hosts; Pitts et al. 2005) or of distantly related genera as in *Megalomyrmex* ants (on hosts in the tribe Attini) (Brandão 1990).

Megalomyrmex (Tribe: Solenopsidini) species are found from southern Mexico to Argentina (Brandão 1990). The genus currently comprises 31 described species and has been divided into four species groups (*silvestrii*, *pusillus*, *leoninus*, and *modestus*) based on taxonomic characters and behavior (Brandão 1990; Brandão 2003). Eight *Megalomyrmex* species, classified in the *silvestrii* species group, are thought to be associated with fungus-growing ant hosts (Tribe: Attini) (Brandão 1990; Introduction Chapter). The attine ants form an obligate mutualism with their specific fungal cultivar strain(s), feeding, protecting, and dispersing it in exchange for nutrients (Currie & Stuart 2001; Little et al. 2003; Quinlan & Cherrett 1979; Silva et al. 2003; Weber 1972). The garden represents a large food resource susceptible to consumption by other organisms through usurpation and parasitism (Adams et al. 2000; Currie & Stuart 2001).

Megalomyrmex wettereri colonies are agro-predators and xenobiotic social parasites (Introduction Chapter). In their agro-predatory state they aggressively attack attine host ants (*Cyphomyrmex longiscapus* and *Mycocepurus* sp.), driving them out or killing them to usurp their gardens (Adams et al. 2000; Adams unpublished). In this strategy the fungus garden is not supplied with new substrate; therefore *M. wettereri* needs to usurp new gardens regularly, as *M. wettereri* appears unable to live independently of attine gardens (Adams et al. 2000). *Megalomyrmex wettereri* has also been observed once living with a *Trachymyrmex cornetzi* colony, suggesting that it may adopt a parasitic strategy depending on the particular host association (Brandão 2003). *Megalomyrmex mondabora* ants are lestobiotic social parasites and *M. symmetochus* ants are xenobiotic social parasites. All three species consume the host garden and brood (Adams & Longino 2007; see Chapters 1 & 2). These *Megalomyrmex* species are currently thought to associate with several host species, spanning both the basal and derived attine genera (Adams & Dewitz 2006; Adams & Longino 2007; Brandão 2003; Introduction Chapter; but see Chapter 3).

All *Megalomyrmex* species found outside the *silvestrii* species group are assumed to be predatory and some have been observed attacking and consuming large and small prey items (Brandão 1990; Adams unpublished). Several of these species also tend homopteran insects, consuming homopteran-produced honeydew while protecting the homoptera (Brandão 1990; Adams unpublished).

Using molecular phylogenetics, we test Brandão's subdivision of the genus *Megalomyrmex* into four species groups (Brandão 1990) and assess congruence between morphological and molecular character information. Using our phylogeny, we also test Darwin's Predation Hypothesis (Darwin 1859), originally proposed for slavemaker ant species that raid and enslave other ant colonies, which postulates that social parasitism evolves from ancestral predatory states.

MATERIALS AND METHODS

Taxon sampling

We include DNA sequence information from a total of 49 *Megalomyrmex* specimens representing 18 ingroup species (Tables 4.1 & 4.2). Of 12 outgroup species, eight belong to the tribe Solenopsidini, two to the tribe Formicoxenini, one to the tribe Myrmicariini, and one to the tribe Meranoplini. All outgroup species belong to genera that were either historically placed in the tribe Solenopsidini or appear to be close relatives to the solenopsidines, based on previous phylogenetic studies (Brady et al. 2006; Moreau et al. 2006), with the exception of the genus *Meranoplus* (tribe Meranoplini).

DNA isolation

Genomic DNA was extracted from a specimens preserved in 99.5% ethanol (Table 4.1). Voucher specimens have been deposited at the National Museum of Natural History,

Smithsonian Institution, Washington, DC, and the Museu de Zoologia da Universidade de São Paulo.

DNA was usually extracted from an entire adult or immature (larva, pupa) specimen. In some cases, partial ants were used (one leg, legs and thorax, head). Specimens were placed in a vial and submerged in liquid nitrogen, then ground into powder with a Teflon pestle. DNA was extracted using the DNeasy™ Tissue Kit (Qiagen Inc., Valencia, CA), with the exception of the final TE buffer volume used for elution. The elution volume ranged from 100 µl to 200 µl, depending on the amount of ant tissue used for the extraction.

Polymerase chain reaction (PCR) amplification

Fragments of two genes, nuclear wingless (wgls) (Ward & Downie 2005) and mitochondrial cytochrome oxidase I (COI) (Kronauer et al. 2004; Simon et al. 1994; T. Schultz Pers. comm.) were amplified. PCR reactions typically contained 1 µl 10X buffer, 1 µl 25 mM $MgCl_2$, 1 µl dNTPs, 1 µl of each primer, 0.8 µl of 100x BSA, and either 0.05 µl TaKaRa Ex Taq with 0.4 µl of extracted DNA or 0.03 µl Promega Taq with 1 µl of extracted DNA. The final volume was brought up to 10 µl with water. The primers used to amplify and sequence these genes are Wg578F/Wg1032R for the wgls gene and CI13F/CI14R, CI21F/24R, and JerryF/BenR for COI (Table 4.3). The amplification conditions for wgls began with an initial denaturation at 95 ºC for 1 min, followed by 40 cycles of 95 ºC for 30 sec, 54.3 ºC for 30 sec, 72 ºC for 90 sec, and a final 3 min elongation at 72 ºC. If the above protocol produced multiple bands, the samples were amplified again with the same protocol and PCR conditions but with a 55 ºC annealing temperature. The amplification conditions for the COI gene began with a denaturation at 94 ºC for 2 min, followed by 30 cycles of 94 ºC for 40 sec, 45 ºC for 60 sec, and 72 ºC for 90 sec, and a final elongation at 72 ºC for 6 min. For samples that did not amplify with the above protocol, a nested PCR was used. Initially, CI13F/CI24R was amplified

using the above PCR conditions using 1 µl of extracted DNA. Then a second PCR was seeded using the first PCR product with the CI13/CI14, JerryF/BenR, and/or CI21F/CI24R primers. The second PCR protocol was adjusted by changing the amplification temperature to 48 ºC and increasing the final elongation time to 10 minutes. All PCR products were checked visually by 1.5% agarose gel electrophoresis. Successful amplifications were cleaned for cycle sequencing using ExoSAP®.

Cycle Sequencing

A 10 µl cycle sequencing reaction was performed using 1ul ABI PRISM® Big Dye™ (Applied Biosystems Inc., Foster City, CA), 0.5 µl half dye, 1 µl5x buffer (400 mM Tris at pH 9.0 and 10 mM $MgCl_2$), and 1 µl of the same PCR primers (Table 4.3). The remainder of the mixture was composed of ultra pure water and 5-200 ng of PCR product template. All samples were sequenced using forward and reverse primers separately. Cycle sequencing conditions began with denaturation at 96 ºC for 2 min, followed by 24 cycles of 96 ºC for 10 sec, 50 ºC for 5 sec, and 60 ºC for 4 min. Products were further cleaned using Sephadex™ in preparation for visualization on an ABI PRISM® 3100 Genetic Analyzer.

Sequence alignment

We successfully amplified 314 bp of wgls and 907 bp of COI for 61 taxa. Sequences were initially edited and assembled into contigs in SeqMan 5.05© (1989-2002 DNASTAR Inc.). Sequences were aligned in BioEdit Sequencing Alignment Editor© (1997-2007) (Hall 1999) using ClustalX 1.9a 160, then again in MacClade 4.06. Both genes are protein-encoding; amino acid sequences therefore could be used to independently confirm alignment (Maddison & Maddison 2003). Single mutations or ambiguous base calls were examined and changed to a more conservative state at each alignment (e.g. if a base appeared as two different nucleotides a code suggesting this was used).

Phylogenetic Analysis

Relationships between species were inferred using several methods of phylogenetic analysis implemented in GARLI v0.95 (Zwickl 2006) and MrBayes v3.1.1 (Huelsenbeck & Ronquist 2001). Taxa with a small percentage of missing data were included in the analyses.

Maximum-likelihood analyses were conducted using GARLI, a program which employs the General Time Reversible (GTR+I+G) model of nucleotide substitution. Separate ML analyses of each of the gene partitions were performed (results not shown) as well as a combined analysis of the wgls and COI gene fragments (Figure 4.1). Each analysis was repeated ten times and the tree with the best likelihood score was identified. To assess the confidence at each node, ML bootstrap analyses consisting of 100 pseudoreplicates were conducted in GARLI.

Bayesian analyses were conducted using MrBayes v3.1.1 (Huelsenbeck & Ronquist 2001). Data were partitioned in Bayesian analyses, with model parameters determined using MrModel Test (Nylander 2004). Data were modeled as six partitions corresponding to codon positions within each gene under the nucleotide model of evolution using the following models: COI: 1^{st}, 2^{nd} and 3^{rd} position = GTR+I+G; Wgls: 1^{st} position = K80+G, 2^{nd} position = SYM+I, and 3^{rd} position = GTR+G. The analysis (6-pB), consisting of 10 million generations.

RESULTS AND DISCUSSION

Phylogenetic analyses recovered the ant genus *Megalomyrmex* as a monophyletic group within the tribe Solenopsidini. Support for monophyly was strong in the 6-partition nucleotide-model Bayesian (6-pB) analysis (Bayesian posterior probability (bpp): 100%) (Figure 4.2). Maximum-likelihood (ML) analyses likewise recovered *Megalomyrmex* as

monophyletic, but this result was only weakly supported by bootstrap (bs) analyses (bs: <50%) (Figure 4.1).

Testing Brandão's (1990) species group classification

DNA sequence data provide an independent test of Brandão's species groups, which are based on morphology. In fact, the molecular data mostly agree with Brandão's species groups (Figures 4.1 & 4.2). Limited taxon sampling prevents us from confidently testing the monophyly of the *modestus* and *pusillus* groups. Obtaining sequence data from more conserved gene regions would help to increase resolution of divergences near the base of the tree.

Silvestrii species group

In the 6-pB analysis, the *silvestrii* group taxa formed a monophyletic group (bpp: 79%) (Figure 4.2); whereas the *silvestrii* group was recovered as paraphyletic in the ML analysis (bs: <50%) (Figure 4.1). Taxon 11, *M. drifti*, is a free-living *pusillus* group species that is positioned between two leaf-litter-dwelling taxa (107 *M*. sp. 1 and *M silvestrii*). The *silvestrii* and *pusillus* species groups are morphologically very similar and the integration of these two groups would not be surprising (Brandão personal communication).

Pusillus species group

Only two species from the *pusillus* group were included, one likely a new species. In the 6-pB analysis, this group is paraphyletic because it includes taxon 68 *M. cyendyra* (68), a member of the *leoninus* species group (bpp: 44%) (Figure 4.2). In contrast, in the ML analysis, taxon 68 does not group with the two *pusillus* group species, instead taxon 75 *M*. sp. nov. (RMMA) is sister to the *M. silvestrii* clade (*silvestrii* group) (bs: <50%) (Figure 4.1).

Modestus species group

Like the *pusillus* group, the *modestus* group is severely underrepresented in the present study, although representatives of species from Ecuador, Costa Rica, and Brazil are included. In the 6-pB analysis, the *modestus*-group species do not group together, although individuals of the same species from the same site do (Figure 4.2). In the ML analysis, the *M. modestus* individuals from Costa Rica and Ecuador are recovered as sister taxa, but this result was only weakly supported (bs: <50%) (Figure 4.1).

Leoninus species group

Excluding taxon 68, the *leoninus* species group is found to be monophyletic in both ML and Bayesian analyses, although the support is weak (bs: <50% & bpp: 58%, respectively) (Figures 4.1 & 4.2). Taxon 68 may be represented by a paralog of the targeted COI gene region because, after multiple repetitions of the PCR and cycle sequencing reactions, it became apparent that there were two different sequences amplifying for taxon 68. These sequences both aligned with the other taxa in the data set but the longer sequence of the two was eventually chosen for use in the analysis. This is likely the explanation for taxon 68 grouping weakly with the *pusillus* group and for the low support for the *leoninus* clade. In future analyses, the ortholog for taxon 68 will be obtained.

Figure 4.1: Single maximum-likelihood (ML) tree inferred with GARLI under the GTR+I+G model. Values near branches represent ML bootstrap support values over 50. Taxa are named first with a sample code, the species name, parasite host (following a backslash if appropriate), and the site and country code (see Table 4.1). In two cases, samples are named *M*. sp. nov. followed by the original collectors initials in parentheses. Taxa for which sequence information was obtained from GenBank are labeled by the accession number (see also Table 4.2). Species groups are indicated by different colored boxes: light green = *silvestrii* species group; pink = *pusillus* species group; yellow = *modestus* species group; blue = *leoninus* species group. The following abbreviations are used for host genera and host status: Serico. = *Sericomyrmex*, Trachy. = *Trachymyrmex*, Aptero. = *Apterostigma*, Cypho. = *Cyphomyrmex*, No host present = Colony was found in a usurped host cavity, Free-liv. = colony was not found in association with a host.

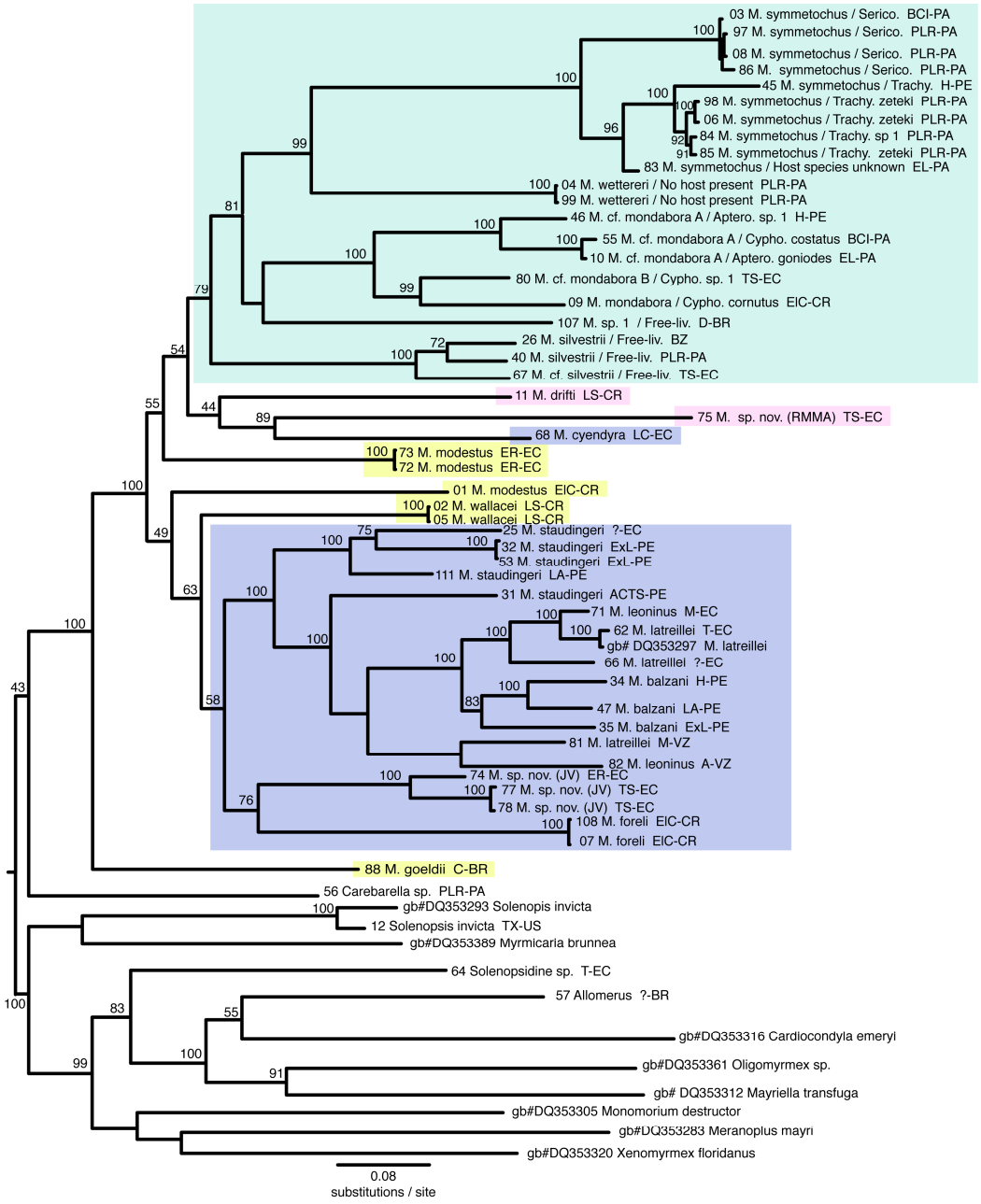

Figure 4.2: Consensus of two converged Bayesian runs partitioned by codon position and gene using the appropriate model (COI: 1st, 2nd and 3rd position = GTR+I+G; wgls: 1st position = K80+G, 2nd position = SYM+I, and 3rd position = GTR+G). Values near branches represent Bayesian posterior probabilities. Taxa are named first with a sample code, the species name, parasite host (following a backslash if appropriate), and the site and country code (see Table 4.1). In two cases, samples are named *M*. sp. nov. followed by the original collectors initials in parentheses. Taxa for which sequence information was obtained from GenBank are labeled by the accession number (see also Table 4.2). Species groups are indicated by different colored boxes: light green = *silvestrii* species group; pink = *pusillus* species group; yellow = *modestus* species group; blue = *leoninus* species group. The following abbreviations are used for host genera and host status: Serico. = *Sericomyrmex*, Trachy. = *Trachymyrmex*, Aptero. = *Apterostigma*, Cypho. = *Cyphomyrmex*, No host present = Colony was found in a usurped host cavity, Free-liv. = colony was not found in association with a host.

Evolution of social parasitism

The *Megalomyrmex* species associated with the Attini are recovered as a monophyletic group in the ML analysis (bs: <50%) (Figure 4.1) and as paraphyletic in the 6-pB analysis, with taxon 107 sister to the *M. mondabora sensu latu* clade (bpp: 81%) (Figure 4.2 & 4.3). These results are consistent with a single origin of social parasitism followed by a possible loss in taxon 107. The attine associates form two distinct clades. The first clade contains two xenobiotic social parasites, *M. symmetochus* and *M. wettereri* (bs: 58% and bpp: 99%) (Figure 4.3). *Megalomyrmex wettereri* is reconstructed as sister to the two *M. symmetochus* clades. The monophyletic *M. symmetochus* clade (bs: 100% and bpp: 100%) is divided further into groups that correspond to host genera. The *Sericomyrmex* species associates are monophyletic (bs: 100% and bpp: 100%) and the

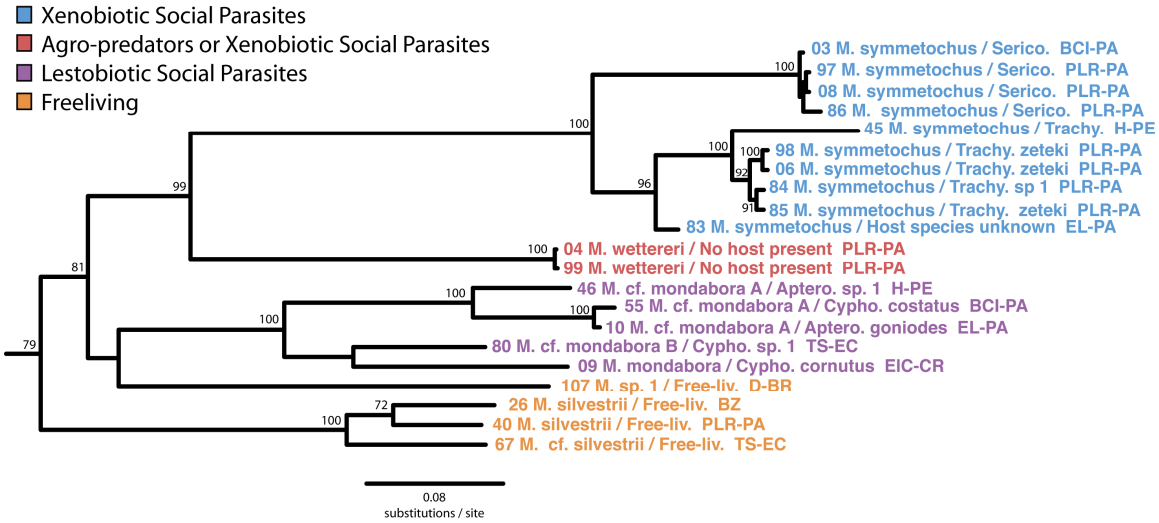

Figure 4.3: The *silvestrii* species group from Figure 2. Taxa font colors indicate natural history characters. The top nine taxa (red) are xenobiotic social parasites. The two taxa in orange font, sister to the top clade, are agro-predators or xenobiotic parasites. The clade with six taxa in green font are lestobiotic social parasites, with the exception of taxon 107. The four taxa in purple font (including taxon 107), are free-living predatory species. The following abbreviations are used for host genera and host status: Serico. = *Sericomyrmex*, Trachy. = *Trachymyrmex*, Aptero. = *Apterostigma*, Cypho. = *Cyphomyrmex*, No host present = Colony was found in a usurped host cavity, Free-liv. = colony was not found in association with a host.

Trachymyrmex species associates are monophyletic (bs: 79% and bpp: 96%), with one taxon collected in Peru while all others are from the same or nearby sites in Panama. These two clades suggest that *M. symmetochus* may encompass two cryptic species, living sympatrically but associating with different host genera.

It had been previously proposed that *M. wettereri,* the agro-predator and xenobiotic social parasite (its life style apparently depending on which host species it is associated with), represents an intermediate stage leading to the most derived behavioral state of social parasitism (Adams et al. 2000). Under this hypothesis we would expect *M. wettereri* to occupy a position basal to (i.e., as the sister group of) the strictly socially parasitic clade consisting of *M. symmetochus* and *M. mondabora s.l.* However, *M. wettereri* does not occupy such a position, nor do *M. symmetochus* and *M. mondabora* form a clade exclusive of *M. wettereri*. Alternatively, therefore the *M. wettereri* agro-predatory lifestyle appears to be derived from an ancestral parasitic state (Adams et al. 2000). This would be the most parsimonious reconstruction of behavioral evolution in the *silvestrii* species group. Given that some *M. wettereri* colonies are xenobiotic parasites, it is probable that either 1) the agro-predatory forms are recently evolved or 2) the appearance of "agro-predatory behavior" is simply a host response to the presence of a parasite. In other words, the behavior of *M. wettereri* is perceived as "predatory-like" because the host abandons its nest. This strategy is also seen in the host species of some bird brood parasites (Seredio & Hauber 2006). In some cases it is less costly for the host to rebuild a nest and lay another clutch of eggs than to remain with a parasitized nest (Servedio & Hauber 2006). *Megalomyrmex wettereri's* host *Cyphomyrmex longiscapus,* are driven from or abandon their nests, fleeing with a portion of their garden. After the host colony builds a new cavity and stabliizes, it may be able to recover most or all of their potential fitness lost if they were to stay in their original cavity and be parasitized by *M. wettereri* ants.

The lestobiotic social parasites, *M. mondabora s. l.*, also form a clade in both analyses (bs: 99% and bpp: 100%). It has been proposed that this clade is probably a species complex (Adams & Longino 2007) and additional data support this view (see Chapter 3). Morphological characters corroborate the well-supported clades dividing the *M. mondabora s. l.* complex into *M*. cf. *mondabora* A (bs: 100% and bpp: 100%) and *M*. *mondabora s. s.* and *M*. cf. *mondabora* B (bs: 79% and bpp: 99%) (Chapter 3).

Testing Darwin's hypothesis of a predatory origin of social parasitism

Our phylogenic results support Darwin's hypothesis that social parasitism in *Megalomyrmex* originated from a predatory ancestor that evolved into a social-parasitic lineage. Specifically, in the ML analysis, the fungus-growing-ant-associated *Megalomyrmex* species form a derived monophyletic group that arise from within a clade of free-living predators (bs: <50%) (Figure 4.1). In the 6-pB analysis, the fungus-growing-ant-associates are paraphyletic (bpp: 81%) but still remain in a derived position in respect to all other *Megalomyrmex* species which are free-living predators (Figures 4.2 & 4.3).

In conclusion, the phylogenetic results 1) prompt numerous questions regarding species delineation in the currently recognized *M. symmetochus* and *M. mondabora* lineages (Brandão 1990; Brandão 2003; Chapter 3); 2) generally support previously proposed species groups based on morphology; and 3) recover the social parasites as a derived monophyletic group emerging from the ancestral predatory species, supporting Darwin's Predation Hypothesis.

Sample ID Collector	Species name	Collection Locality, Country (code)	DNA code	Host
22.iv.2001-01 **?. Tarores**	*M. goeldii* (det. R. F. Brandão)	Cunha, Brazil (CB)	88	none
22b1 Die47 **unknown**	*M. staudingeri* (det. R. F. Brandão)	Ecuador (?-EC)	25	none
AGH030212-13 **A. G. Himler**	*M. mondabora* (det. R. F. Brandão & J. T. Longino)	El Ceibo, Costa Rica (ElC-CR)	9	*C. cornutus* (det. J. T. Longino)
AMG031210-02 **A. M. Green**	*M. symmetochus*	El Llano, Panama (ElL-PA)	83	*Trachymyrmex* sp.
B070497-12 **L. R. Davis, Jr.**	*M. silvestrii* (det. R. F. Brandão & J. T. Longino)	? Belize	26	unknown
CR040529-06 **C. Rabeling**	*M. symmetochus*	Huacaria, Peru (H-PE)	45	*Trachymyrmex* sp. 1
DD502 **D. Donoso**	*M. latreillei*	Tiputini, Ecuador (T-EC)	62	none
DD546 **D. Donoso**	Solenopsidini Tribe	Tiputini, Ecuador (T-EC)	64	none
HF010330-50 **H. Fernandez-Marin**	*M. mondabora* (det. R. F. Brandão) *M.* cf. *mondabora* A (det.J. T. Longino)	El Llano, Panama (ElL-PA)	10	*Apterostigma goniodes* (det. J. T. Longino)
HV **H. Vasconcelos**	*Allomerus*	Brazil (?-BR)	57	none
JMV-PB025 **J. Vieira**	*M. latreillei*	Ecuador (?-EC)	66	none
JMV-TO12 **J. Vieira**	*M.* cf. silvestrii	Toachi Station, Ecuador (TS-EC)	67	unknown
KER031212-02 **K. E. R.**	*M. symmetochus*	Barro Colorado Island, Panama (BCI-PA)	3	*Sericomyrmex* sp.
NMG011030-02 **N. M. Gerardo**	*M. symmetochus*	Pipeline Rd., Panama (PLR-PA)	97	*Sericomyrmex* sp.
RC1 **R. Cárdenas**	*M. cyendyra*	Los Cedros, Ecuador (LC-EC)	68	none
RMMA000818-01 **R. M. M. Adams**	*M.* sp. 1	Dimona site, Brazil (D-BR)	107	none
RMMA030214-02 **R. M. M. Adams**	*M. foreli* (det. R. F. Brandão)	El Ceibo, Costa Rica (ElC-CR)	7	none
RMMA030811-01 **R. M. M. Adams**	*M. modestus* (det. R. F. Brandão)	El Ceibo, Costa Rica (ElC-CR)	1	none
RMMA030819-07 **R. M. M. Adams**	*M. wallacei* (det. R. F. Brandão)	La Selva, Costa Rica (LS-CR)	2	none
RMMA030819-08 **R. M. M. Adams**	*M. wallacei*	La Selva, Costa Rica (LS-CR)	5	none
RMMA040315-01 **R. M. M. Adams**	*S. invicta*	Austin, TX USA (TX-US)	12	none
RMMA040528-08 **R. M. M. Adams**	*M.* cf. *mondabora* A (det. J. T. Longino)	Huacaria, Peru (H-PE)	46	*Apterostigma* sp.
RMMA040529-01 **R. M. M. Adams**	*M. balzani*	Huacaria, Peru (H-PE)	34	none
RMMA040604-01 **R. M. M. Adams**	*M. staudingeri* (det. R. F. Brandão)	Los Amigos, Peru (LA-PE)	111	none
RMMA040609-05 **R. M. M. Adams**	*M. balzani* (det. R. F. Brandão)	Los Amigos, Peru (LA-PE)	48	none
RMMA040612-02 **R. M. M. Adams**	*M. balzani*	Explorama Lodge, Peru (ExL-PE)	35	none
RMMA040613-05 **R. M. M. Adams**	*M. staudingeri* (det. R. F. Brandão)	Explorama Lodge, Peru (ExL-PE)	32	none
RMMA040614-12 **R. M. M. Adams**	*M. staudingeri* (det. R. F. Brandão)	Explorama Lodge, Peru (ExL-PE)	53	none

RMMA040618-04 R. M. M. Adams	*M*. sp. 2 (det. R. F. Brandão)	Explorama ACTS, Peru (ExL-PE)	31	none
RMMA041223-04 R. M. M. Adams	*M*. cf. *mondabora* A (det. J. T. Longino)	Barro Colorado Island, Panama (BCI-PA)	55	*Cyphomyrmex costatus*
RMMA041230-17 R. M. M. Adams	*Carebarella* sp. (det. C. Rabeling)	Pipeline Rd., Panama (PLR-PA)	56	none
RMMA050626-01 R. M. M. Adams	*M. foreli*	El Ceibo, Costa Rica (ElC-CR)	108	none
RMMA050729-12 R. M. M. Adams	*M. symmetochus* (det. J. T. Longino)	Pipeline Rd., Panama (PLR-PA)	86	*Sericomyrmex* sp. 1
RMMA050801-08 R. M. M. Adams	*M. symmetochus*	Pipeline Rd., Panama (PLR-PA)	85	*Trachymyrmex zeteki*
RMMA050818-05 R. M. M. Adams	*M. symmetochus*	Pipeline Rd., Panama (PLR-PA)	84	*Trachymyrmex* cf. zeteki 2
RMMA060304-20 R. M. M. Adams	*M. leoninus* (det. R. M. M. Adams)	Misahuallí, Ecuador (M-EC)	71	none
RMMA060307-14 R. M. M. Adams	*M. modestus*	Endesa Reserve, Ecuador (ER-EC)	72	none
RMMA060308-02 R. M. M. Adams	*M. modestus*	Endesa Reserve, Ecuador (ER-EC)	73	none
RMMA060308-03 R. M. M. Adams	*M*. sp. nov. (JV) (det. J. Vieira)	Endesa Reserve, Ecuador (ER-EC)	74	none
RMMA060311-15 R. M. M. Adams	*M*. sp. 1 (RMMA)	Toachi Station, Ecuador (TS-EC)	75	none
RMMA060312-02 R. M. M. Adams	*M*. sp. nov. (JV) (det. J. Vieira)	Toachi Station, Ecuador (TS-EC)	77	none
RMMA060313-22 R. M. M. Adams	*M*. sp. nov. (JV) (det. J. Vieira)	Toachi Station, Ecuador (TS-EC)	78	none
RMMA060315-02 R. M. M. Adams	*M*. cf. *mondabora* B (det. J. T. Longino)	Toachi Station, Ecuador (TS-EC)	80	*Cyphomyrmex* (*rimosus* group)
RMMA990929-06 R. M. M. Adams	*M. symmetochus* (det. Brandão)	Pipeline Rd., Panama (PLR-PA)	6	*Trachymyrmex*
RMMA990930-07 R. M. M. Adams	*M. silvestrii* (det. R. F. Brandão & J. T. Longino)	Pipeline Rd., Panama (PLR-PA)	40	none
RMMA990930-19 R. M. M. Adams	*M. symmetochus* (det. R. F. Brandão)	Pipeline Rd., Panama (PLR-PA)	98	*Trachymyrmex*
3VIII05 S. Solomon	*M. latreillei* ?	Merida, Venezuela (M-VE)	81	none
SES050730-02 S. E. Solomon	*M. leoninus*?	Aragua, Venezuela (A-VE)	82	none
tpm297335 Terry McGlynn	*M. drifti* (det. R. F. Brandão)	La Selva, Costa Rica (LS-CR)	11	none
?UGM010321-28? unknown	*M. symmetochus* (det. J. T. Longino)	Pipeline Rd., Panama (PLR-PA)	8	*Sericomyrmex* sp. 1
UGM010327-25 U. G. Mueller	*M. wettereri* (det. R. F. Brandão)	Pipeline Rd., Panama (PLR-PA)	4	none present
UGM011205-08 U. G. Mueller	*M. wettereri* (det. R. F. Brandão)	Pipeline Rd., Panama (PLR-PA)	99	none present

Table 4.1: Individuals included in the phylogenetic analyses. DNA code refers to the number before the species name on the phylogenies. In the host column, "none" indicates that the respective *Megalomyrmex* species is not known to associate with or was not collected with a host; "none present" indicates that no attine host was present at the time of collection because the respective *Megalomyrmex* species is an agro-predator that eliminates its host during the invasion; "unknown" means that the *Megalomyrmex silvestrii* species has been recorded being collected with a host (Weber 1941) but collection information for this particular sample gave no information of the biology of the colony and was likely collected using leaf-litter sampling methods. In all other cases, we list the host species with which the respective *Megalomyrmex* species was associated at the time of collection.

Species Name & Voucher ID	Locus	GenBank #
Cardiocondyla emeryi RA0330	COI	DQ353316
	wingless	DQ353021
Mayriella transfuga RA0265	COI	DQ353312
	wingless	DQ353052
Megalomyrmex latreillei CS0330	COI	DQ353297
	wingless	DQ353054
Meranoplus mayri CS0249	COI	DQ353283
	wingless	DQ353006
Monomorium destructor RA0242	COI	DQ353305
	wingless	DQ353012
Myrmicaria brunnea RA0283	COI	DQ353389
	wingless	DQ353014
***Oligomyrmex* sp.** CSM-2006	COI	DQ353361
	wingless	DQ353047
Solenopsis invicta CASENT0500523	COI	DQ353293
	wingless	DQ353039
Xenomyrmex floridanus RA0340	COI	DQ353320
	wingless	DQ353005

Table 4.2: Sequence information for taxa obtained from GenBank and originally published by Moreau et al. (2006).

Gene and Primer Name	Sequence (5' to 3')	Citation
COI: Jerry forward	CAACATTTATTTTGATTTTTTGG	Simon et al. (1994)
COI: Ben reverse	GCTACTACATAATAKGTATCATG	T. Schultz pers. comm.
COI: CI13 forward	ATAATTTTTTTTATAGTTATACC	Simon et al. (1994)
COI: CI14 reverse	GTTTCTTTTTTTCCTCTTC	Simon et al. (1994)
COI: CI21 forward	CTTTATCAACATTTATTTTGAT	Simon et al. (1994)
COI: CI24 reverse	TCCTAAAAAATGTTGAGGAAA	Simon et al. (1994)
wgls: 578 forward	TGCACNGTGAARACYTGCTGGATGCG	Ward and Downie (2005)
wgls: 1032 reverse	ACYTCGCAGCACCARTGGAA	Ward and Downie (2005)

Table 4.3: Primer sequences used to amplify and sequence fragments of the Cytochrome Oxidase I (COI) and nuclear wingless (wgls) genes.

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Appendices:

APPENDIX A: VENOM ALKALOIDS OF *MEGALOMYRMEX* ANTS

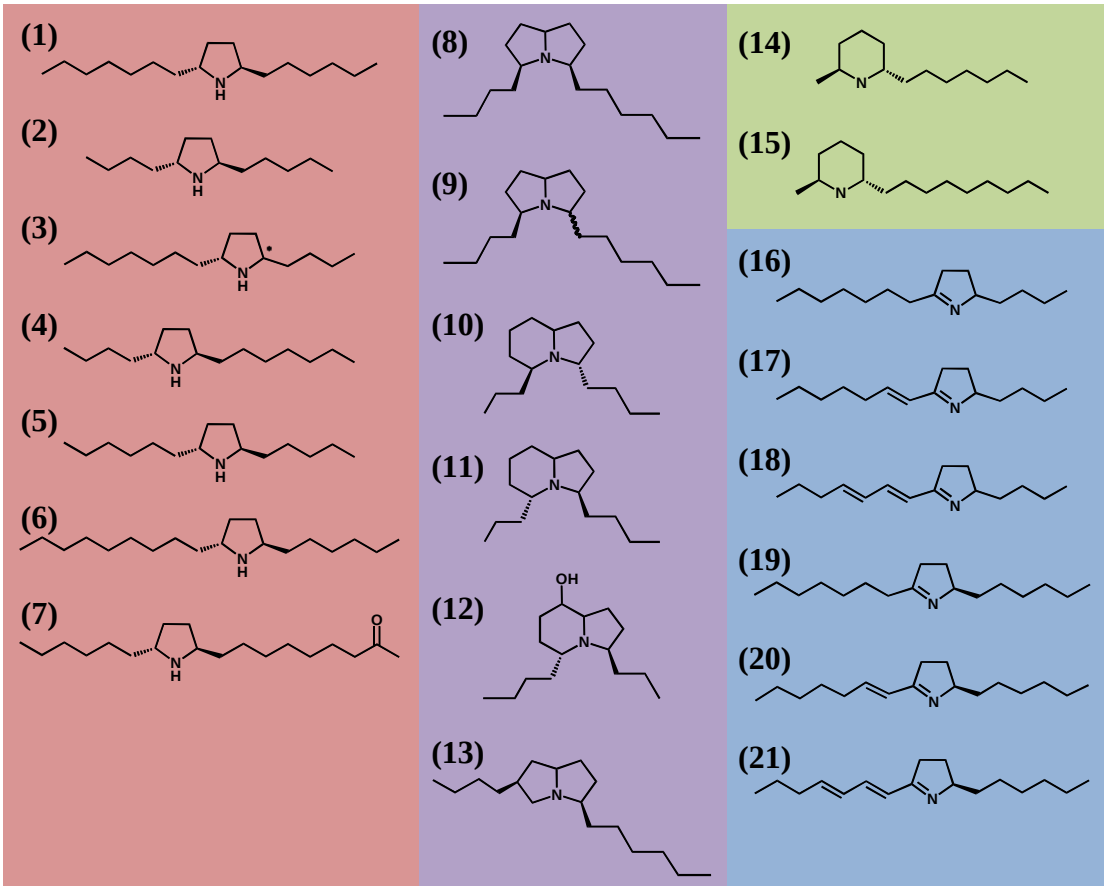

Table A1: Alkaloids Identified from *Megalomyrmex* Species. [a] ++= major component; + = minor component (5-20%); o = trace (< 1%); "empty cell" = not detected; x = present but amount unknown

Venom Alkaloids of *Megalomyrmex*																							
***Megalomyrmex* species**	**Collection and DNA number Collection site**	**Pyrrolidine**							**Pyrrolizidine**						**Piperidine**		**Pyrroline**						**Citations**
		trans-2-heptyl-5-hexylpyrrolidine	*trans*-2-butyl-5-pentylpyrrolidine	*trans*-2-butyl-5-heptylpyrrolidine	*trans*-2-butyl-5-heptylpyrrolidine	*trans*-2-hexyl-5-pentylpyrrolidine	*trans*-2-nonyl-5-hexylpyrrolidine	*trans*-2-hexyl-5-[8-oxononyl]-pyrrolidine	*cis*-2-butyl-8-hexylpyrrolizidine	*cis/trans*-2-butyl-8-hexylpyrrolizidine	*trans*-2-butyl-8-propylindolizidine	*trans*-2-butyl-8-propylindolizidine	*trans*-2-butyl-8-propyl-4-hydroxyindolizidine	(5E,8E)-3-butyl-8-hexlypyrrolizidine	*trans*-2-methyl-6-heptylpiperidine	*trans*-2-methyl-6-nonylpiperidine	2-butyl-5-heptyl-5-pyrroline	2-butyl-5-(E, 1-heptenyl)-5-pyrroline	2-butyl-5-(E,E, 1,3-heptadienyl)-5-pyrroline	2-hexyl-5-heptyl-5-pyrroline	2- hexyl -5-(E, 1-heptenyl)-5-pyrroline	2- hexyl -5-(E,E, 1,3-heptadienyl)-5-pyrroline	
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
***leoninus* Species Group**																							
M. balzani	RMMA040529-01 (DNA 34,44) Huacaria, Peru				+ +																		present study
M. balzani	RMMA040609-05 Los Amigos, Peru				+ +																		present study

M. balzani	RMMA040612-02 Explorama Lodge, Peru				+ +																		present study
M.* cf. *balzani	RMMA040614-07 Peru				+ +																		present study
M.* cf. *balzani	RMMA040614-12 (DNA 53) Explorama Lodge, Peru				+ +																		present study
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. cyendyra		o			+ +																		(Jones *et al*. 1999)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M.* cf. *emeryi / staudingeri	RMMA040616-06 Peru	+ +																					present study
M.* cf. *emeryi / staudingeri	RMMA040618-04 (DNA 31, 54) Explorama ACTS, Peru	+ +																					present study Also other ialkaloids
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. foreli	RMMA030214-02 (1) Aug. 03 col. El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli	RMMA030214-02 (2) Aug. 03 col. El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli	RMMA030214-02 El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli	RMMA030812-03 El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli	RMMA030812-08 El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli	RMMA030812-09 El Ceibo, Costa Rica	+																		+	+	+	present study

M. foreli	RMMA030812-10 El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli	RMMA050627-05 El Ceibo, Costa Rica	+																		+	+	+	present study
M. foreli				+													+ +	+ +	+ +				(Jones *et al*. 1991b)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. latreillei		+ +		+ +																			(Jones *et al*. 1999)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. leoninus	(worker)	+ +																					(Jones *et al*. 1991a)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. staudingeri	RMMA040604-01 (DNA 33, 111) Los Amigos, Peru				+ +																		present study
M. staudingeri	RMMA040613-05 (male) (DNA 32) Explorama Lodge, Peru																						present study/ None
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
***modestus* Species Group**																							
M. goeldii	(worker)	+ +	o	+ +		o																	(Jones *et al*. 1991a)
M. goeldii	(wingless queens)	o	+ +	o		+ +																	(Jones *et al*. 1991a)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. modestus	RMMA030212-08 El Ceibo, Costa Rica	o			+ +																		present study
M. modestus	RMMA030810-01 Costa Rica	o			+ +																		present study
M. modestus	RMMA030810-02 Costa Rica	o			+ +																		present study
M. modestus	RMMA030811-01.1 (DNA 1) El Ceibo, Costa Rica	o			+ +																		present study

M. modestus	RMMA030811-01.2 (DNA 1) El Ceibo, Costa Rica	o			+ +																		present study
M. modestus	RMMA030811-01.3 (DNA 1) El Ceibo, Costa Rica	o			+ +																		present study
M. modestus	RMMA030811-01 (DNA 1) in Meth. Chlor. El Ceibo, Costa Rica	o			+ +																		present study
M. modestus	RMMA030811-03 Costa Rica	o			+ +																		present study
M. modestus	RMMA030811-04 Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-01 Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-02 (1) Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-02 (3) (males) Costa Rica																						present study/ none
M. modestus	RMMA 030812-02 (2) contaminated Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-04 A Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-04 B Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-05 Costa Rica	o			+ +																		present study
M. modestus	RMMA 30812-06 Costa Rica	o			+ +																		present study
M. modestus	RMMA 030812-07 Costa Rica	o			+ +																		present study
M. modestus	RMMA 030818-02 Costa Rica	o			+ +																		present study
M. modestus	RMMA050624-03 La Selva, Costa Rica	o			+ +																		present study

M. modestus	RMMA050624-03 (worker-produced male) La Selva, Costa Rica																						present study/ none
M. modestus	RMMA050624-04 Costa Rica	o			+ +																		present study
M. modestus	RMMA 050707-01 (gaster) La Selva, Costa Rica	o			+ +																		present study
M. modestus	RMMA 050707-01 (head) La Selva, Costa Rica				o																		present study
M. modestus	RMMA 050707-01 (thorax) La Selva, Costa Rica				o																		present study
M. modestus	RMMA 050707-01 (whole ant) La Selva, Costa Rica	o			+ +																		present study
M.* cf. *modestus	RMMA050810-06 El Llano, Panama	o			+ +																		present study
M. modestus														+ +									(Jones *et al.* 1991a)
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. wallacei	RMMA030819-02 La Selva, Costa Rica					+ +																	present study
M. wallacei	RMMA030819-04 (male) La Selva, Costa Rica					+ +																	present study
M. wallacei	RMMA030819-06 La Selva, Costa Rica					+ +																	present study
M. wallacei	RMMA030819-06 (male) La Selva, Costa Rica																						present study/ none

M. wallacei	RMMA030819-07 (DNA 2) La Selva, Costa Rica					+ +																	present study
M. wallacei	RMMA030819-08 (DNA 5) La Selva, Costa Rica					+ +																	present study
M. wallacei	RMMA030819-08 (male) (DNA 5) La Selva, Costa Rica																						present study/ none
M. wallacei	RMMA030819-10 La Selva, Costa Rica					+ +																	present study
M. wallacei	July 2005 (gaster) La Selva, Costa Rica					+ +																	present study
M. wallacei	July 2005 (head) La Selva, Costa Rica																						present study/ none
M. wallacei	July 2005 (thorax) La Selva, Costa Rica																						present study/ none
M. wallacei	RMMA050704-03 (male) La Selva, Costa Rica																						present study/ none
M. wallacei	RMMA050704-03 (wingless queen) La Selva, Costa Rica					+ +																	present study
M. wallacei	RMMA050704-04 (male) La Selva, Costa Rica																						present study/ none
M. wallacei	RMMA050710-01 (male) La Selva, Costa Rica																						present study/ none
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
***silvestrii* Species Group**																							
M. mondabora s.s.	RMMA030213-09 El Ceibo, Costa Rica	+ +					o	+ +															Chapter 3

M. mondabora s.s.	RMMA030213-07 El Ceibo, Costa Rica	+ +					o	+ +															Chapter 3
M. mondabora s.s.	RMMA050625-01 El Ceibo, Costa Rica	+ +					o	+ +															Chapter 3
***M.* cf. *mondabora* A**	HF010330-50 El Llano, Panama														+	+ +							Chapter 3
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
***M. symmetochus* with *Trachy* host**	RMMA990930-19 (DNA 98) PLR, Panama								+		+	+	+										present study/ also some dibutyl-pyrrolizidine
***M. symmetochus* with *Serico* host**	RMMA050729-12 B (male) (1) (DNA 86) PLR, Panama																						present study/ none
***M. symmetochus* with *Serico* host**	RMMA050729-12 B (male) (2) (DNA 86) PLR, Panama																						present study/ none
***M. symmetochus* with *Serico* host**	RMMA050729-12 B (male) (3) (DNA 86) PLR, Panama																						present study/ none
***M. symmetochus* with *Trachy* sp. 1 (big red) host**	RMMA050818-05 (w) PLR, Panama										+	+	+										present study/ also some dibutyl-pyrrolizidine
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
M. wettereri	RMMA990922-14 PLR, Panama								+ +														present study/ also a isomer of 6

M. wettereri	RMMA050808-02 PLR, Panama								x	+ +													present study/ also isomers of 6 or 7
M. wettereri	RMMA050808-02 (male) PLR, Panama																						present study/ none
M. wettereri	RMMA050808-02 (male) PLR, Panama																						present study/ none
		1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	
***M. sp* 1**	**RMMA040608-06 Peru**	o			+ +																		present study

APPENDIX B: FUTURE PERSPECTIVES

The molecular phylogenetics of the ant tribe Solenopsidini

The ant tribe Solenopsidini comprises eleven genera containing 1,041 species and subspecies. Many are involved in symbiotic relationships with plants, fungi, and ants. Some of these associations are mutualistic, while an unusually large number are socially parasitic. The social parasites exploit other ant colonies, negatively impacting the host's fitness (i.e., survival or reproduction). They may parasitize close relatives within the tribe or distantly related species belonging to other tribes. Social parasites of the fire ant *Solenopsis invicta* are of particular interest because they are potential biological control agents. The fire ant is one of several invasive species in the tribe that are responsible for great economic and ecological damage and have thus been the subject of much research.

In addition to social parasites, the tribe Solenopsidini contains an unusually large number of species with alternative reproductive strategies. Rather than producing typical winged queens, colonies rely on wingless female reproductives to begin new colonies. Both social parasites and wingless queens have evolved multiple times within the tribe suggesting a predisposition to such evolutionary trajectories. Using comparative methods and molecular phylogenetics, I will examine the evolution of both traits and test two evolutionary hypotheses, one proposed by Darwin (1859) and one by Bolton (1986). Darwin's hypothesis suggests that social parasitism originates when a formerly predatory species evolves into a social parasite. Bolton's hypothesis suggests that winglessness in queens is correlated with a change in nest-founding behavior from claustral founding (independent founding by queens) to founding by fission (where the newly mated queen returns to her natal nest, leaving with a subset of her mother's work force). These hypotheses will be tested by including species that exhibit these characteristics as well as their suggested sister species, based on morphology, in a molecular phylogenetic study using eight genes.

Although the tribe Solenopsidini has over the years received a fair amount of attention from ant systematists, there is little agreement about tribal-level relationships. Great progress has been made in the differentiating species, but it remains unclear what genera belong in the tribe. Thus, in addition to answering evolutionary questions, I will help to resolve some of the taxonomic challenges of this tribe.

Chemical Ecology of *Megalomyrmex* Social Parasites

The interdisciplinary study of chemical communication has developed into a cutting-edge field of science that can address key questions on the organization of life at both the cellular and the organismal level. The study of communication in insect societies and their social parasites has played a pivotal role in these developments. However, suitable model lineages with a sufficient number of evolutionary transitions between normal social living and social parasitism are uncommon and even fewer have been thoroughly studied. This proposal intends to make such a key model system available for study. *Megalomyrmex* ants are chemical warriors, dispensing volatile venom alkaloids (VAs) by waving their stings (i.e. gaster flagging) as they enter another ant species' nest as parasites or during competition (by predatory free-living species). Species of both lifestyles use gaster flagging as a 'warning shot', announcing their presence to hosts or competitors. If the host does not allow infiltration, the invader will attack and kill. Infiltration can be accomplished as just described (i.e. chemical weaponry) or through the alteration of cuticular hydrocarbons (CHCs) (i.e. surface chemistry) using chemical mimicry and/or insignificance. I intend to decipher the chemical code of communication and manipulation of *Megalomyrmex* ants, linking behaviors observed to their chemical ecology. I will 1) test three infiltration strategy hypotheses using behavioral experimentation, 2) identify relevant chemical compounds, and 3) examine the results in an evolutionary context with phylogenetic hypothesis testing. Using the unique *Megalomyrmex* system I pioneered, I plan to gain focused training which will enhance

my job opportunities and future collaborations and encourage a shift in the research on social parasitism towards a more comparative approach.

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Vita

Rachelle Martha Marie Adams was born on September 12th, 1973 in Jerseyville, Illinois, the daughter of Susan and Richard Adams. After graduating from Jersey Community High School, Jerseyville, Illinois, in 1991 she attended Western Illinois University, Macomb, Illinois then transferred to The University of Montana, Missoula, Montana in 1993. There she shifted her focus from horticulture to social insect biology, first, working with honeybees for Jerry Bromenshenk then with bumblebees and Australian halictine bees for Penelope Kukuk. Penelope Kukuk and Steve Forbes introduced Rachelle to molecular techniques that sparked her interest in evolutionary biology. As an undergraduate she was awarded funding by the Honors College to pursue an independent research project on bumblebees. After graduation in May, 1997, Rachelle worked as a technician for Steve Forbes for six months, before moving to the Washington DC area to work with Ted Schultz (Smithsonian Institution) and Ulrich Mueller (University of Maryland, at that time) on a joint project studying the fungus-growing ants. After two years, Rachelle moved to Austin, Texas and continued her technician work with Ulrich Mueller. Soon after the move in September, 1999 she experienced her first tropical field season in Panama, where she further developed her interests in social parasitism and evolutionary biology. In January 2002, Rachelle entered graduate school at The University of Texas at Austin.

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